# Diversity of the genus *Seira* (Collembola: Entomobryidae: Seirinae) in the Fynbos and Southern Afrotemperate forest

by

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**Abstract**

Soil is one of the most complex and understudied terrestrial habitats, and it comprises a wide range of organisms that affect the functioning of terrestrial ecosystems. Despite the importance of these below-ground fauna, our understanding of this diversity remains limited, especially in the Cape Floristic Region (CFR), where higher plants and other more conspicuous invertebrates have been better represented in the literatures. The main aim of this thesis is to provide the first insights into the taxonomic, cryptic and spatial diversity of one of the more diverse Collembola genera, *Seira* (Collembola: Entomobryidae: Seirinae), in the Fynbos and Southern Afrotemperate forest habitats of the CFR. Collembola constitute an important component of terrestrial biodiversity and are essential for ecosystem functioning.

The thesis is divided into two data chapters. The first data chapter examines the mitochondrial barcoding COI (Cytochrome-*c* oxidase subunit I) gene of 496 *Seira* specimens from 41 Fynbos and forest sites. Discrete Molecular Operational Taxonomic Units (MOTUs) are identified within *Seira*. Furthermore, divergence times are estimated and tentatively used to propose historical triggers for the diversification of *Seira*. Habitat specificity of *Seira* is assessed through phylogenetic reconstruction using Parsimony and Bayesian analyses based on the nucleotide and amino acid sequences. Genetic divergence indicates that *Seira* is composed of at least 91 MOTUs, suggesting that morphological taxonomy has vastly under-estimated the richness of this genus by at least four folds. Most of the MOTUs are highly habitat specific and geographically localised. The reconstruction of an evolutionary time frame of these lineages reveals several deep diversifications in the Miocene and a spate of more recent radiations in the Pleistocene and Holocene. Palaeo-environmental fluctuations and vegetation composition shifts are hypothesised as having increased the environmental complexity of the region and this may have influenced the diversification patterns of *Seira.*

In the second data chapter, the spatial variation of *Seira* diversity is examined and compared between and within two major habitat types, Fynbos and forest, across nine study sites. Abiotic variables were recorded and compared for the investigation of environmental differences among habitat types. The habitat specificity of *Seira* is assessed, as well as the relative effects of habitat types and study sites on assemblage structure. Species richness of *Seira* is higher in Fynbos than in forest, and this could be due to the high heterogeneity of niches and resources created by the steep environmental and floristic gradients in the Fynbos habitats. Perhaps one of the most

striking results is the high levels of beta diversity exhibited by *Seira*. The spatial turnover of the *Seira* assemblage is complete or nearly complete among and within habitat types across study sites within the CFR. Low dispersal abilities and consequently isolated evolutionary histories may account for the strong assemblage differences within the same habitat type of different study sites. However, the substantial differences in assemblage composition between adjacent habitat types in the same site are likely to be the result of the contrasting abiotic conditions exhibited by the Fynbos and forest habitats, as found by this study.

Overall, the results of this thesis suggest that *Seira* species richness is much greater than previously thought. It follows similar diversity patterns to the well-described and hyper-diverse plant communities of the CFR. Here, for both CFR plants and *Seira*, the spectacular regional diversity displayed is not the result of high alpha diversity, but rather of substantial beta diversity. Because of the high beta diversity and the fact that only two CFR vegetation types were sampled from a limited number of study sites, I predict that the *Seira* species richness presented here is but the tip of the iceberg.

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“There is no better high than discovery.” - Edward O. Wilson

# Chapter 1

## General introduction

Together with tropical forest canopies, soil has long been recognised as another biotic frontier of the terrestrial biota (André et al., 1994). Soil organisms encompass a range of life-forms and functions, and play crucial roles in ecosystem processes and services (Lavelle et al., 2006). The documentation and understanding of soil fauna diversity is fundamental for conservation research and planning, as changes in their diversity could have potential implications for the continued functioning of terrestrial ecosystems (Decaëns et al., 2006). Globally, soil fauna represents an estimated 23% (circa 360,000) (Decaëns et al., 2006) of the 1.5 million described species on Earth (Costello et al., 2012). Moreover, estimates of 5.5 and 6.8 million species have been proposed for insect and terrestrial arthropod groups, respectively (Stork et al., 2015); which suggests that many soil species still await description. Despite the significant contribution of soil fauna to global biodiversity, they have received much less taxonomic attention than the above-ground biota (Decaëns et al., 2006). It has been highlighted that the taxonomic deficit (the proportion of expected taxa to described taxa) is negatively associated with the body size, and small body-sized fauna constitutes much of the soil biota (Decaëns et al., 2006; Decaëns, 2010). For this reason, only a fraction of the total taxonomic diversity of soil biota has been formally described (Decaëns et al., 2006; Decaëns, 2010). Consequently, our knowledge regarding the quantification and spatial patterns of soil fauna diversity remains limited. The recent increase in DNA-based approaches, either in an integrative framework or as an alternative to traditional methods, has potentially addressed the taxonomic constraints imposed by small body size in a range of biota. This has occurred within a timeframe which would have been impossible to achieve using conventional morphological characters alone (Hebert et al., 2003, 2004a, b; Tautz et al., 2003; Smith et al., 2005; Rougerie et al., 2009; Decaëns et al., 2013; Shaw et al., 2013).

### *Cape Floristic Region*

The Cape Floristic Region (CFR), located at the south-western tip of the African continent, is the smallest of the six floristic kingdoms of the world and harbours one of the most exceptional floral assemblages in terms of diversity and endemism (Good, 1953; Myers et al., 2000). This Mediterranean-like region of about 90 760 km$^2$ contains approximately 9 383 species of vascular plant, and almost 50% of these belong to only 33 Cape floral clades (Linder, 2003). Furthermore, the region has a specific level of endemism of 68.3% (Manning & Goldblatt, 2012). This exceptional level of endemism is thought to be a consequence of combined ecological and

geographical isolation of the CFR, which resemble the conditions typically experienced by island communities rather than continental areas (Linder, 2003; Manning & Goldblatt, 2012).

According to a global environmental model predicting plant species richness developed by Kreft & Jetz (2007), the Cape flora is more than twice as rich in species per unit area as predicted by a range of macro-ecological variables; confirming its remarkable diversity. This raises interesting questions about the scales at which floral diversity is organised in the Cape. It appears that the species diversity is only moderately high at the local scale (alpha diversity), which is not surprising in the context of Mediterranean-climates (Cowling et al., 1992, 1996; Simmons & Cowling, 1996; Goldblatt, 1997; Goldblatt & Manning, 2002). However, the differentiation of species composition between different habitats (beta diversity), and among equivalent habitats along geographical gradients (gamma diversity) is exceptionally high (Cowling et al., 1992; Simmons & Cowling, 1996; Goldblatt, 1997). It is thought that complex climatic, topographic and edaphic conditions may have led to the striking level of floristic diversity, by creating a mosaic of diverse habitats across the entire region (Goldblatt, 1997; Goldblatt & Manning, 2002; Linder, 2003). Additionally, factors limiting gene flow between populations may also have a profound influence on the flora speciation (Linder, 2003). There is a pronounced gradient of decreasing plant species richness and endemism from the south-western to the south-eastern landscape (Cowling & Holmes, 1992; Cowling & Lombard, 2002; Goldblatt & Manning, 2002; Linder, 2003; Cowling & Procheş, 2005); but increasing phylogenetic diversity along this gradient (Cowling, 1983; Forest et al., 2007b). The difference between these two sections in the CFR is thought to be the consequence of varied speciation and extinction rates, promoted by substantial differences in both historical and contemporary environmental conditions (Cowling et al., 1992, 1999, 2009; Cowling & Lombard, 2002; Linder, 2003; Cowling & Procheş, 2005).

The majority of the plant species in the CFR occur in the distinctive vegetation element known as the Fynbos; which is a sclerophyllous, fire-adapted shrubland, occurring mostly within a winter rainfall region (Goldblatt, 1997; Goldblatt & Manning, 2002; Cowling & Pierce, 2004). However the abiotic trait which most strongly characterises Fynbos is that it has nutrient-deprived soil (Bergh et al., 2014). Fynbos covers approximately 55% of the region with an estimated 7 500 species, of which 60% are endemics (Rebelo et al., 2006). It comprises a variety of ancient and recent lineages (Linder, 2005, 2008; Verboom et al., 2014), with the date of initiation of diversification ranging from the Late Eocene-Early Miocene (Goldblatt et al., 2002; Linder &

Hardy, 2004; Forest et al., 2007a; Verboom et al., 2009; Slingsby et al., 2014), to the Mid Miocene-Pliocene (Linder et al., 1992; Richardson et al., 2001; Linder, 2003; Verboom et al., 2003). The radiation during the Late Miocene was accompanied by the marked extinction and contraction of the mesic evergreen forest communities that previously dominated the Cape region (Linder et al., 1992; Dupont et al., 2011). Numerous studies have hypothesised that several environmental phenomena or the interactions between them, including climatic fluctuations, the establishment of Benguela current, tectonic movements, uplift of mountain ranges, edaphic changes, sea-level fluctuations and the onset of the recurrent fire regime, have triggered the diversification of Cape lineages by altering or generating a range of habitats available for flora colonisation in the region (Goldblatt 1997; Richardson et al., 2001; Linder, 2003, 2005; Cowling et al., 2009; Bytebier et al., 2011).

As a consequence of the Cenozoic environmental changes, the fragmented Southern Afrotemperate forest is imbedded in the more widespread contemporary Fynbos Biome (Manders et al., 1992; Rebelo et al., 2006). The forest is known to be relatively plant species-poor at both local and regional scales, although it does support some endemics (Cowling & Holmes, 1992; Mucina & Geldenhuys, 2006). For example, the Western Cape Afrotemperate forests comprise only 48 woody plant species and few endemics (Mucina & Geldenhuys, 2006). In contrast to the Fynbos vegetation, there is a gradual decline in forest taxa richness and endemism, from the extreme southeast to the southwest section of the region (Lawrence, 1953; Verboom et al., 2014); possibly due to the less favourable edaphic and climatic conditions in the southwest (Cowling & Holmes, 1992; Cowling et al., 1992). The forest communities are chemically and structurally resistant to the periodic fires that characterise Fynbos habitats (van Wilgen et al., 1990). As such, fire does not form an integral part of the ecosystem dynamic of the forests, except at their edges (Mucina & Geldenhuys, 2006). Furthermore, in contrast to Fynbos, the soil surface layers of the forest are associated with high nutrient availability, which is attributed to the variety of fallen litter types and the death of forest fauna (Mucina & Geldenhuys, 2006). The plant-induced effects coupled with different nutrient recycling processes of the organic materials may have resulted in the differences in soil chemicals between the Fynbos and forest habitats (Manders et al., 1992). Therefore, the Fynbos and Southern Afrotemperate forest vegetation are governed by different biotic and abiotic variables and thus have very little in common with respect of their floristic species composition (Campbell & Moll, 1977; Bergh et al., 2014) and community processes (Manders & Richardson, 1992); likely affecting the invertebrate communities associated with each of these habitat types.

*Invertebrate studies in the CFR*

Despite their dominance in terms of global biodiversity and importance in ecosystem functioning (Gascon et al., 2015; Stork et al., 2015), studies on the terrestrial invertebrate diversity patterns are scarce in the CFR due to a paucity of taxonomic expertise (Picker & Samways, 1996). Based on the available studies, there is much dispute relating to the level of invertebrate diversity in the CFR, with Giliomee (2003) suggesting the insect richness of the region was not particularly high in comparison with other areas in South Africa. However, this idea was not supported in some studies of plant specialists (i.e. the gall-forming insects; Wright & Samways, 1998) or pollinators (Colville, 2009). Furthermore, diversity of a broad range of other insect groups (Procheş & Cowling, 2006; Procheş et al., 2009) was positively associated with plant species richness in the CFR. Procheş et al. (2009) suggested that the relationship between plant and insect diversity at large spatial scales was possibly the result of both taxonomic groups responding similarly to the same environmental conditions. However, at fine spatial scales, they suggested that the relationship was the result of direct causality. It must be noted that positive associations between plant and insect species richness do not necessarily mean that insect diversity in the CFR is exceptionally high. In fact, a global comparison with similar habitats found that the local richness of ant species in the CFR was neither exceptionally rich nor poor in the Fynbos and Succulent Karoo biomes, and that patterns of species diversity matched those which were predicted based on climate and available energy (Braschler et al., 2012). Procheş & Cowling (2006) suggested that invertebrate diversity in Fynbos was comparable to, or higher than, the other selected neighbouring South African biomes at both local and broader spatial scales. Therefore, although previous perceptions of exceptionally low insect species richness in the CFR (Giliomee, 2003) may be incorrect, there is no evidence to suggest that the insect richness is an exceptional latitudinal outlier as it is with plant species richness.

Several species richness comparisons between forest and Fynbos have been conducted in the Cape Peninsula. Pryke & Samways (2010) found that the richness and abundance of surface-active and aerial invertebrate species was higher in Fynbos than in forest sites (only the foliage invertebrate diversity appeared to be somewhat higher in the forest; Pryke & Samways, 2008). Patterns of higher invertebrate richness in the Fynbos could be a result of increased species turnover, promoted by the heterogeneous environment associated with the Fynbos landscape (Pryke & Samways, 2008, 2010). Although the forests generally held fewer species of invertebrates, these relictual habitats do appear to be particularly rich in invertebrate endemics of ancient origin (Picker & Samways, 1996; Ratsirarson et al., 2002; Pryke & Samways, 2009a,

2010; Foord et al., 2011). Moreover, there were clear distinctions between the invertebrate assemblages occupying the Fynbos and Southern Afrotemperate forest habitats (Koen & Breytenbach, 1988; Pryke & Samways, 2008, 2009b, 2010), which also differ considerably in their biotic- and abiotic-related variables (Campbell & Moll, 1977; Manders & Richardson, 1992; Manders et al., 1992;). The Fynbos-inhabiting invertebrate fauna appeared to be highly adapted to the locally arid environment, while species occupying forest habitats are adapted to the more humid environment (Pryke & Samways, 2010).

The studies in faunal evolutionary history of the CFR have, to a great extent, been vertebrate focused (e.g. Smit et al., 2007; Swart et al., 2009; Tolley et al., 2009; du Toit et al., 2012; Oatley et al., 2012). However, recent years have witnessed increasing research effort using phylogenetic approaches directed at some of the terrestrial invertebrate taxa in the region (e.g. Price et al., 2007; McDonald & Daniels, 2012; Sole & Scholtz, 2013; Sole et al., 2013). Based on the molecular divergence time estimate, it was shown that the diversification of terrestrial invertebrates was associated with the Miocene/Pliocene/Pleistocene (Price et al., 2007, 2010; Daniels et al., 2009, 2013; McDonald & Daniels, 2012; Sole et al., 2005, 2013; Sole & Scholtz, 2013), a dynamic time of combined geomorphic and climatic changes, and the shift from ecological dominance of forests to Cape shrubland taxa (Linder et al., 1992; Linder, 2003; Cowling et al., 2009). Consequently these environmental perturbations have been implicated as the drivers of invertebrate cladogenesis in the CFR (Price et al., 2007; Daniels et al., 2009; McDonald & Daniels, 2012). Several close phylogenetic relationships were found between the velvet worm taxa inhabiting isolated Afromontane forest patches, which suggest the historical connection between these forests in the region (McDonald & Daniels, 2012; Daniels et al., 2009, 2013).

*Collembola*

While the number of insect diversity and molecular studies in the CFR are continuously increasing (e.g. Pryke & Samways, 2008, 2010; Colville, 2009; Procheş et al., 2009; Braschler et al., 2012), the inconspicuous mesofaunal soil communities such as the class Collembola have remained comparatively understudied with few recently published works (Liu et al., 2012; Janion et al., 2011b; Janion-Scheepers et al., 2015). The Collembola (springtails) are one of the more ancient (Hirst & Maulik, 1926; Whalley & Jarzembowski, 1981; Misof et al., 2014) and abundant groups of arthropods which occur in most terrestrial ecosystems globally (Hopkin, 1997; Rusek, 1998; Deharveng, 2004). Collembola have a reported global estimate of 8 500 described species

(Bellinger et al., 2015) and a projected estimate of 65 000 species (Deharveng, 2004; Porco et al., 2014). There are four main orders, namely Poduromorpha, Entomobryomorpha, Symphypleona and Neelipleona (Hopkin, 1997). Their functional significance in both below- and above-ground ecosystem processes are widely acknowledged (Wardle et al., 2004; De Deyn & Van der Putten, 2005; Bardgett & Van der Putten, 2014), as well as their roles as bioindicators for the ecological integrity of habitats due to their sensitivity to environmental changes (Lawrence, 1953; Hopkin, 1997; van Straalen, 1998; Chagnon et al., 2001; Zeppelini et al., 2009). Furthermore, they appear to play a functional role in the litter decomposition and nutrient cycling in the CFR soil systems (Bengtsson et al. 2011, 2012; Leinaas et al., 2015).

Progress has been made recently concerning the Collembola taxonomy in South Africa, with an increase in newly described species (Janion et al., 2011a, 2012, 2013; Potapov et al., 2011). A total of 160 species have been recorded in South Africa, of which 65% are endemic to the country and 67 of which are known to occur in the Western Cape Province (Janion-Scheepers et al., 2015). The level of endemism is comparable to that of the flora and other invertebrate fauna in South Africa, especially in the south-western Cape (see plants: Rebelo et al., 2006; Manning & Goldblatt, 2012; see invertebrates: Picker & Samways, 1996; Colville et al., 2002; Pryke & Samways, 2010). Furthermore, data from the well-sampled Western Cape indicated that the overall species richness could be at least six to seven times higher than what is currently known in the literature (Janion-Scheepers et al., 2015).

Although diversity patterns of Collembola are poorly known in the CFR, spatial diversity patterns and their correlates have attracted a great deal of research in other regions of the world, mostly from the Northern Hemisphere (e.g. Deharveng, 1996; Vanbergen et al., 2007; Robson et al., 2009; Ulrich & Fiera, 2009; Fiera & Ulrich, 2012; Heiniger et al., 2014). I know of only one local scale study addressing these questions at the species level in the CFR (Liu et al., 2012). The spatial diversity patterns of Collembola are often influenced by several chemical and physical edaphic parameters (Cassagne et al., 2003; Vangergen et al., 2007; Raschmanová et al., 2008, 2013; Robson et al., 2009; Salamon & Alphei, 2009; Nielsen et al., 2010; Zeppelini et al., 2013; Heiniger et al., 2014). Other abiotic and biotic aspects of the habitat, such as temperature, humidity, heterogeneity, plant diversity and litter, have also been found to have effects on the Collembolan assemblages (Vangergen et al., 2007; Robson et al., 2009; Sabais et al., 2011; Liu

et al., 2012). Some of these responses of Collembola assemblages were scale- and species-dependent.

The use of molecular tools in both species discovery and identification process has increased tremendously over the past decade as a possible solution to the overwhelming taxonomic task for the Collembola group (Hebert et al., 2003; Hogg & Hebert, 2004; Rougerie et al., 2009). Based on the mitochondrial barcoding COI (Cytochrome-*c* oxidase subunit I) gene, recent studies in different parts of the world (e.g. Europe, Canada and Antarctica) have revealed remarkable levels of DNA sequence divergence amongst morphologically defined Collembola lineages; which most likely indicate the presence of distinct genetic entities or cryptic species (Hogg & Hebert, 2004; Rougerie et al., 2009; Porco et al., 2010, 2014; Emerson et al., 2011; Katz et al., 2015). These results were in agreement with a slower evolving nuclear DNA marker (Porco et al., 2012a, b). Furthermore, persistent localisation and genetic isolation over a substantial period is commonly reported in Collembola (Garrick et al., 2004, 2007; Timmermans et al., 2005; Stevens et al., 2007; Cicconardi et al., 2010, 2013; Torricelli et al., 2010). In general, the underestimation of true global species richness is perhaps due to the existence of pervasive cryptic species (e.g. Deharveng, 2004; Torricelli et al., 2010; Emerson et al., 2011; Porco et al., 2014), along with strong taxonomic impediment (Decaëns, 2010) and geographical under-sampling (Hopkin, 1997).

*This thesis*

The genus *Seira* forms part of one of the most diverse families of Collembola, the Entomobryidae (Christiansen & Bellinger, 2000). It seems to be ubiquitous in most terrestrial habitats globally, with the exception of Antarctica (Christiansen & Bellinger, 2000). There are around 205 described species grouped into three subgenera (Bellinger et al., 2015), and new species are constantly being described elsewhere in the world (e.g. Christiansen & Bellinger, 2000; Bellini & Zeppelini, 2009, 2011; Godeiro & Bellini, 2014; Cipola et al., 2014) due to the continuous advances in Collembola systematics (Rusek, 1998; Deharveng, 2004). In South Africa, 29 *Seira* species have been described (Börner, 1908; Womersley, 1934; Paclt, 1959, 1967), of which 21 are recognised for the Western Cape (Yosii 1959; Coates, 1968). However, recent literature on the South African Collembola, based on both morphological and DNA barcoding approaches, have indicated significant cryptic diversity (Porco et al., 2012b) and it has been suggested that this genus is undergoing a major radiation in the CFR (Janion et al., 2011b), together with other genera, such

as *Parisotoma* (Potapov et al., 2011) and *Ectonura* (Janion et al., 2011a). Therefore, it is essential to understand the diversity of this genus and its spatial patterns across the hyper-diverse CFR.

In an attempt to address the dearth of knowledge regarding this understudied group of terrestrial mesofauna in the CFR, the main aim of this study is to provide the first insight into the diversity and differences between the *Seira* species in the pristine Fynbos and Southern Afrotemperate forest habitats across the region. This was carried out through comprehensive sampling using commonly used techniques (i.e. litter collection, beating above-ground vegetation) (e.g. Deharveng, 1996; Rusek, 1998; Janion et al., 2011b) in both habitat types. In chapter two, a DNA barcode system from a standardised portion of the mitochondrial COI gene (Hebert et al., 2003; Hogg & Hebert, 2004; Ratnasingham & Hebert, 2007) was used to discover and quantify species richness and the extent of morphologically cryptic species in the *Seira* lineages. Here, divergence times were tentatively used to suggest triggers for *Seira* diversification. In chapter three, the spatial diversity of *Seira* in the contrasting habitat types were compared and described through three traditionally recognised components, namely the alpha, beta and gamma diversity (Whittaker, 1960, 1972).

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## Chapter 2

## Diversity estimates of genus *Seira* (Collembola: Entomobryidae: Seirinae) in the Fynbos and Southern Afrotemperate forest of Cape Floristic Region using a single mitochondrial barcoding gene


### Abstract

The Cape Floristic Region (CFR) is known for its exceptional plant diversity where most radiations appear to have occurred within only a few plant lineages. In contrast, the diversity and radiations of invertebrate taxa in the region, especially the invertebrates occurring in the soil ecosystem, has remained understudied. This study is the first assessment of the taxonomic and cryptic diversity within the genus *Seira* (Collembola: Entomobryidae: Seirinae), a group of mesofaunal arthropods with 21 known species in the Western Cape. Sampling was conducted over 41 Fynbos and Southern Afrotemperate forest sites across the CFR. The sequence information of 496 *Seira* specimens from the mitochondrial barcoding COI (Cytochrome-*c* oxidase subunit I) gene is analysed, and discrete Molecular Operational Taxonomic Units (MOTUs) are delineated. Divergence times are estimated using published mutation rates for arthropods, and are tentatively used to propose historical triggers for the diversification of *Seira*. Habitat specificity of *Seira* is assessed through phylogenetic reconstruction using Parsimony and Bayesian analyses based on the nucleotide and amino acid sequences. This study has revealed striking richness of *Seira* where 91 MOTUs are identified. Of these, 63 are uniquely linked to the Fynbos and 20 are only found in the forest habitats, with deep sequence divergence among them. Eight MOTUs were identified as habitat generalists. Eighty of the 91 MOTUs are identified morphologically, indicating the presence of cryptic species. This suggests that the current *Seira* taxonomy is likely an underestimation of the true *Seira* species richness within the CFR. In addition, the mitochondrial COI gene supports previous studies proving its effectiveness in species-level discovery and revealing morphologically cryptic taxa. A large proportion of the MOTUs are both geographically localised and habitat specific, which may indicate strong ecological structuring. The reconstruction of an evolutionary time frame of these lineages reveals several deep diversifications in the Miocene and a spate of more recent radiations in the Pleistocene and Holocene. Palaeo-environmental fluctuations and vegetation composition shifts are hypothesised as having increased the environmental complexity of the region and this may have influenced the diversification patterns of *Seira.*

## Introduction

Molecular analyses have transformed our understanding of the impact of historical and evolutionary processes on the assembly of contemporary biotic systems (Epperson, 2003; Lowe et al., 2004; Taberlet et al., 2012). Patterns of diversity are characterised by the regional accumulation of species through diversification, extinction, immigration, and the ecological sorting of species within an area (Ricklefs, 2007). By combining molecular phylogenies with ecological and geographical information, much insight into these processes can be gained (Linder, 2005; Barraclough, 2006). Molecular phylogenetic information is especially useful for the historical reconstruction of assemblage development, where fossil evidence is sparse. The Cape Floristic Region (CFR) in South Africa is one such case (Linder, 2003).

The CFR is renowned for its exceptional concentration of plant diversity and endemism, and has long been recognised as one of the six global floral kingdoms (Good, 1953; Myers et al., 2000). The majority of the richness and endemism occurs in the distinctive Fynbos vegetation, which covers 55% of the region (Manning & Goldblatt, 2012). Thus, the CFR has been ideal for evolutionary studies attempting to understand the historical events that triggered the origin of this remarkable floral diversity, as well as the period over which diversification occurred (e.g. Levyns, 1964; Linder et al., 1992; Richardson et al., 2001; Linder, 2003; Cowling et al., 2009). The diversification of several Cape lineages have been associated with the gradual aridification of the region as a result of the formation of the nutrient-rich cold Benguela upwelling system in the Late Miocene, as well as the increased topographic and edaphic heterogeneity in the CFR (Richardson et al., 2001; Linder, 2003; Verboom et al., 2003; Cowling et al., 2009; Dupont et al., 2011). Climatic fluctuations and the onset of recurrent fire regimes are thought to have precipitated a shift in the ecological dominance of mesic-adapted forest elements to the more fire-prone and arid-adapted Cape shrubland lineages in the Middle Miocene (Linder et al., 1992; Linder, 2003; Bytebier et al., 2011).

Evidence has revealed that the initial radiations of several Cape plant lineages are relatively ancient, dating back to the Eocene, Oligocene and Early Miocene (Goldblatt et al., 2002; Linder & Hardy, 2004; Forest et al., 2007; Verboom et al., 2009; Slingsby et al., 2014), when the paleoclimate was thought to be characterised by warmer, more humid and aseasonal rainfall regimes than presently (Deacon et al., 1992; Partridge, 1997a; Linder, 2003). The sandstones of the Cape Fold Mountains potentially provided stable refugia for the long-term persistence of Fynbos vegetation (Verboom et al., 2009). The present-day Cape floral diversity

has been attributed to the relatively stable climate during the Pleistocene, which allowed diversification to continue throughout this period with relatively low rates of extinction, particularly in the western part of the CFR (Dynesius & Jansson, 2000; Midgley et al., 2001; Cowling & Lombard, 2002; Latimer et al., 2005). The western CFR has been identified as an area of remarkable floral diversification for the majority of the plant clades, comprising evolutionary closely related taxa; while the eastern part is recognised as an area of modest diversification with notably lower numbers of species and less endemism, but comprising a mixture of lineages from various biomes (Verboom et al., 2014).

Although the evolutionary history of plants has been extensively investigated in the CFR, similar studies for terrestrial invertebrates in the region are less common (but see Price et al., 2007; Daniels et al., 2009, 2013; McDonald & Daniels, 2012; Sole & Scholtz, 2013; Sole et al., 2013). It has been suggested that the speciation of velvet worms was mainly associated with the Early Miocene, a time marked by climatic and geological amelioration (Daniels et al., 2009). More recent diversifications were reported from the Pliocene/Pleistocene boundary in several invertebrate groups (Sole et al., 2005; Price et al., 2007, 2010; McDonald & Daniels, 2012). It has been implied that there once was a historical connection between the existing isolated Afromontane forest patches in the CFR, including the north-western and southern Cape, the south-western Cape and the Cape Peninsula (Daniels et al., 2009, 2013). A combination of geomorphic and climatic changes during the Miocene and Early Pliocene significantly contracted and isolated forest patches (Cowling et al., 2009), and may have restricted the forest-living invertebrates to high-elevation refugia (Daniels et al., 2009). It has been suggested that there is a diverse group of paleo-endemic invertebrate groups of high conservation importance inhabiting the vegetation types present at high elevation areas in the Cape Peninsula, such as the Afrotemperate forests and Fynbos (Picker & Samways, 1996; Colville et al., 2014). Within the CFR, the low-lying Cape Flats has been identified as a barrier to the dispersal of some terrestrial and aquatic invertebrate lineages, as it isolates the Cape Peninsula from other mountain blocks of the Cape Fold Belt, such as the Hottentots-Holland (Wishart & Hughes, 2003; Gouws et al., 2004; McDonald & Daniels, 2012; Daniels et al., 2013). Moreover, high levels of genetic differentiation were found between the invertebrate populations of the southwestern and eastern sections of the region (Price et al., 2007; Damgaard et al., 2008; Daniels et al., 2009; Sole & Scholtz, 2013).

Despite their significant contribution to diversity and soil ecosystem services (Lawrence, 1953; Wardle et al., 2004; De Deyn & Van der Putten, 2005; Bardgett & Van der Putten, 2014), soil

invertebrate communities in the CFR have remained poorly explored. Collembola represent one of the more ancient (Hirst & Maulik, 1926; Whalley & Jarzembowski, 1981; Misof et al., 2014) and abundant groups of mesofaunal arthropods in most terrestrial ecosystems globally (Hopkin, 1997; Rusek, 1998; Deharveng, 2004). This class has a reported global richness of approximately 8 500 described species (Bellinger et al., 2015) and a projected global estimate of 65 000 species (Deharveng, 2004; Porco et al., 2014). Collembola, though diverse in the CFR and comprising several speciose genera, lend themselves particularly to an molecular-based approach because of recent work undertaken to understand the distribution and extent of their diversity in southern Africa (Janion et al., 2011b; Janion-Scheepers et al., 2015).

The genus *Seira* (Lubbock, 1870) belongs to one of the most diverse families of Collembola, namely the Entomobryidae, with 205 described species grouped in three subgenera (Bellinger et al., 2015). The genus appears to be ubiquitous in terrestrial habitats, with the exception of Antarctica (Christiansen & Bellinger, 2000). *Seira* is predominately concentrated in Africa and tropical America (Christiansen & Bellinger, 2000), and appears to show significant radiation in southern Africa (Yosii, 1959; Coates, 1968). Recent studies of Collembola in South Africa, coupling morphological and DNA approaches, have revealed significant cryptic diversity (Porco et al., 2012b), with notable radiations in genera such as *Parisotoma* (Potapov et al., 2011), *Ectonura* (Janion et al., 2011a) and *Seira* (Janion et al., 2011b). Twenty-nine species of *Seira* have been described in South Africa (Börner, 1908; Womersley, 1934; Paclt, 1959, 1967; Yosii, 1959), 21 of which are known to occur in the Western Cape (Coates, 1968). However, Janion et al. (2011b) suggests that this is likely to be an underestimation of the actual richness.

Conservation planning requires some *a priori* knowledge regarding the number of species present in an area. This knowledge may also provide preliminary insights into the likely processes that have given rise to the currently observed patterns of diversity. The overall aim of this study is therefore to provide a first assessment of the taxonomic diversity within the genus *Seira*, with a focus on the differences in species composition between Fynbos and Southern Afrotemperate forest habitats in the CFR. For this, a genetic approach was implemented where unique Molecular Operational Taxonomic Units (MOTUs; Floyd et al., 2002; Blaxter, 2004), which may correspond to species, were identified based on their genetic distinctness. The specific objectives of the study were (1) to estimate the species richness of the genus *Seira* in the Fynbos and forest habitats of the CFR by following a DNA barcoding approach (Hebert et al., 2003b; Hogg & Hebert, 2004; Porco et al., 2012b, 2014), tree-based

criteria and nucleotide pairwise divergence thresholds; (2) to determine whether these taxonomic groups are restricted to a given habitat type; (3) to determine the extent to which *Seira* species richness is composed of cryptic species (this is done in combination with Chapter 3); and (4) to provide a preliminary estimate of time of divergence based on the known COI substitution rates for arthropods, and to consider the possible historical triggers for the diversification of this genus in the two habitat types.

## Materials and methods

*Taxon sampling*

Samples were collected from a total of 41 sampling sites (23 sites in the Fynbos and 18 in the Southern Afrotemperate forest) across the CFR between 2008 and 2013 (Figure 2.1; Table 2.1) by the *Protea I* and *Protea II* projects, a joint research endeavour between researchers at the Centre for Invasion Biology in South Africa and the Museum National d'Histoire Naturelle in France (Janion et al., 2011b). Samples were collected from nine geographically separate areas, and Fynbos and forest habitats were sampled in each area where possible. Forest habitat was not represented in four of the nine areas and Fynbos habitat was not represented in one of the nine areas. The western and eastern regions of the CFR differ both topographically and climatically (Linder, 2003). The south-western part is characterized by a Mediterranean climate, while the eastern region lies in a summer-dominated rainfall area (Goldblatt, 1978; Linder, 2003; Rebelo et al., 2006). Rainfall is non-seasonal along the south coast, with maximum precipitation in spring and autumn (Linder, 2003; Rebelo et al., 2006). The region is predominately comprised of the fire-prone Fynbos vegetation and, to a lesser extent, the fire-sensitive indigenous Southern Afrotemperate forest remnants (Mucina & Geldenhuys, 2006; Rebelo et al., 2006).

To maximize species discovery, individuals were collected via two commonly used sampling techniques: litter collection and above-ground shrub beating (e.g. Deharveng, 1996; Rusek, 1998; Janion et al., 2011b). GPS coordinates were recorded for the location of each sampled point (See Table 2.1 for collection details). Specimens were collected using an aspirator with vegetation beating and/or extracted from the litter, soil or rotten wood samples using the Berlese-Tullgren funnel extraction method (Hopkin, 2007; Liu et al., 2012). Specimen extraction was carried out as soon as possible after collection to minimize natural deaths and the risk of being preyed on by other arthropods such as spiders, mites and beetles (Rusek, 1998; Hopkin, 2007). Extractions of specimens were done over five to seven days or until the sample was dry. Specimens were preserved in absolute ethanol under cool conditions, and subsequently sorted under a Leica MZ7.5 dissection microscope in the laboratory. Given that taxonomic keys are available for the 29 known *Seira* species (Börner, 1908; Womersley, 1934; Coates, 1968; Paclt, 1959, 1967; Yosii, 1959; Bellinger et al., 2015), these were initially used for the sorting of specimens into morphospecies. However, it became clear that the potential number of species was much higher than suggested from the literature, and specimens were subsequently separated into morphospecies based on their external characteristics (e.g. colour/pigment patterns; see Supplementary Figure S1). Reference material collected under

the *Protea I* and *Protea II* projects was also consulted. The samples were sorted by various researchers (including myself) and staff at the Centre for Invasion Biology. However, to ensure identification consistency, the final morphospecies identification was done jointly by C. Janion-Scheepers and I. Samples were stored in 96-99% ethanol at the Molecular Zoology Laboratory, University of Johannesburg, South Africa, the Centre of Invasion Biology, University of Stellenbosch, South Africa, and the Museum National d'Histoire Naturelle (MNHN), Paris, France.

*Laboratory protocols and treatment of sequence data*

Specimens collected were barcoded as part of the COLSA (Collembola of South Africa) project, but also as part of the larger BCCOL (Barcoding Collembola) campaign, which aims to create a barcoding library for all Collembola species. BCCOL is linked to the Barcoding of Life Data (BOLD) systems, which is an online tool "*aiding the acquisition, storage, analysis and publication of DNA barcode records*" (Ratnasingham & Hebert, 2007). Protocols followed those prescribed by BOLD (Ratnasingham & Hebert, 2007). A random subset of individuals per identified morphospecies from each sampled site was chosen for DNA barcoding. Where possible and as a general rule, a minimum of four individuals per morphospecies per site were sequenced. These specimens were individually photographed for morphological vouchering using a Leica MZ7.5 stereo microscope and a Leica DFC320 digital camera. Specimens were then transferred to the uniquely barcoded 96-well glass fibre plates (95 specimens, one control) with absolute ethanol. The plates were sent to the Biodiversity Institute of Ontario, Guelph, Canada for DNA extraction and sequencing (www.boldsystems.org).

The full DNA extraction protocol is available at www.ccdb.ca/docs/CCDB_DNA_Extraction.pdf. In short, extractions were carried out in a 30 µL final volume containing lysis buffer and proteinase K. Following overnight incubation at 56 °C, the DNA was isolated in glass fibre plates (Ivanova et al., 2006). For amplification, M13 tailed primers LCO1490 and HCO2198 (Folmer et al., 1994) were used to target a 658 bp fragment of the 5' end of the mitochondrial COI gene; the COI gene is universally accepted as the animal barcoding gene (Hebert et al., 2003a, b). In cases where the Folmer et al. (1994) primers did not yield amplicons, internal primers were used to amplify shorter but overlapping segments (LepF1-MLepR1 and MLepF1-LepR1; Hajibabaei et al., 2005). Standard polymerase chain reaction (PCR) amplification protocols are available at www.ccdb.ca/docs/CCDB_Amplification.pdf. Successful amplification was verified in 2% E-

gel® 96 Agarose (Invitrogen). The M13 tailed primers were used to sequence the PCR amplicons in both directions. Sequencing reactions were performed using BigDye® chemistry (Applied Biosystems, Warrington, UK). Sequencing products were run on an ABI 3730 DNA Analyser (Applied Biosystems, Warrington, UK). Sequences, photographs, taxonomic as well as collection information of the specimens were deposited in the BOLD Identification System under the COLSA project. After DNA extraction, the recovered exoskeletons of specimens were stored in absolute ethanol for reference, as the chaetotaxy characteristics can be used in further morphological examination when necessary (Porco et al., 2010b).

Forward and reverse sequences were assembled as contigs in Geneious v. 7.1.3. (Biomatters Ltd, Auckland, New Zealand; Drummond et al., 2013). Using the same software, sequences were aligned using either the pairwise or multiple MUSCLE alignment algorithms, followed by manual inspection and editing if necessary.

*MOTU delineation*

Previous studies on Collembola have revealed exceptionally high levels of DNA sequence divergence between recognized taxa, which may indicate the presence of cryptic species within the classical morphologically defined species (see e.g. Hogg & Hebert, 2004; Rougerie et al., 2009; Emerson et al., 2011; Porco et al., 2014; Katz et al., 2015). These high levels of nucleotide divergence are unlikely to be an artefact of the mitochondrial DNA molecule as they have also been similarly reported for nuclear genes (Porco et al., 2012a, b). Although species assignments should never be based on sequence divergences alone, DNA data provide a useful starting point in assessing taxonomic diversity, especially for taxa which are understudied or show little morphological divergence. For Collembola specifically, sequence divergences of 8% and 14% have been proposed for the delimitation of species (Hogg & Hebert, 2004 and Porco et al., 2014, respectively). However, this remains to be robustly tested for a divergent range of taxonomic groups, and values may differ between different taxonomic groups. Inter- and intra-specific divergence values for single Collembola species varied extensively between different taxonomic groups (see e.g. Frati et al., 2000; Hogg & Hebert, 2004; Steven & Hogg, 2006; Stevens et al., 2006; Porco et al., 2010a, 2012a, b, 2014; Emerson et al., 2011; Katz et al., 2015). Keeping these uncertainties in mind, the MOTU delimitation of Cape *Seira* in the present study followed an approach based on both sequence divergence values (including barcoding gap analyses; Meyer & Paulay, 2005), and overall clustering in the tree topology.

In particular, following Collins & Cruickshank (2013), an important distinction was made between species discovery and species identification, as there are major differences between their aims and methodological approaches. In cases where completed or near-completed baseline data are available as reference, the Best Close Match method by Meier et al. (2006) can be followed as the unknown can be compared to known data for identification purposes (species identification). However, this was not possible for the poorly explored *Seira* group in the CFR, and the aim of this study was rather that of species discovery, using DNA barcoding as a species richness assessment tool. For this, a Neighbour-Joining (NJ) tree (Saitou & Nei, 1987) was constructed based on the uncorrected COI sequence divergences values, estimated by the proportion (*p*) of dissimilar nucleotide sites between sequence pairs (in Geneious V. 7.1.3; Biomatters Ltd, Auckland, New Zealand; Drummond et al., 2013). The aim of this approach was to identify unique monophyletic taxonomic groups that are distinct from other such groups. For this approach to work, sequence divergences between groups have to be greater than the variation within groups (the so-called 10 x average intraspecific difference; Hebert et al., 2004). To confirm this was indeed the case, a barcoding gap analysis was conducted in R statistical software 3.1.3 (R Development Core Team, 2015) by means of the *dist.dna*, *sppVector* and *sppDist* function in the 'SPIDER' package 1.3-0 (Brown et al., 2012).

*Habitat assignment*

Sampling information was used for the assignment of each MOTU to a particular habitat (Fynbos or Southern Afrotemperate forest) based on the abundance data from chapter three. Tourist species are transient individuals in a given habitat type without intimate or lasting associations with that particular habitat (Moran & Southwood, 1982). Tourist species can lead to overestimations in the total number of species within habitats (Gaston et al., 1993), thus biasing species occurrence estimates across habitats (Mercer et al., 2000). Therefore, the tourist individuals in this study were identified and corrected for their habitat type association. For criteria in identifying tourist individuals, see chapter three.

*Phylogenetic analyses*

Although the inclusion of only a single mitochondrial gene can be problematic for phylogenetic reconstruction (Funk & Omland, 2003), it can provide some baseline insights into the recent evolutionary history. As a first assessment of whether specific MOTUs or groups of MOTUs are associated with specific habitat types, two parameter-rich approaches were adopted. First, the nucleotide data from the COI gene was analysed in a Parsimony as well as Bayesian

Inference (BI) framework. Secondly, given the high sequence divergence values separating many of the taxa, the data were translated and reduced into the more conservative amino acid codons using MacClade v. 4.08a (Maddison & Maddison, 2005), and it was again analysed in both Parsimony and BI frameworks. The most appropriate DNA substitution model describing the evolution of the COI gene was determined according to the Akaike Information Criterion (AIC) (Akaike, 1974; Posada & Buckley, 2004) as implemented in jModeltest v. 2.1.4 (Guindon & Gascuel, 2003; Darriba et al., 2012). The sister genus to *Seira* remains unknown. Thus, sequences of three Collembola species were included as outgroups representing Entomobryidae (same family), Isotomidae (different family) and Hypogastruridae (different order) (Table 2.1).

To minimize the possibility of tree searches being trapped on local optima, subsets of individuals representing each of the MOTUs were included to reduce the number of swapping events between closely-related individuals; where only one or two specimens comprised a MOTU, all specimens were retained. Bayesian Inference trees (for the nucleotide as well as amino acid datasets) were constructed in MrBayes v. 3.2.2 (Ronquist & Huelsenbeck, 2003). For the nucleotide data, eight Markov Chain Monte Carlo (MCMC) simulations with a heating temperature of 0.006 were run for 20 million generations and a sample was drawn every 5000$^{th}$ generation. The same tuning of the number of MCMC runs and chains was applied for the translated amino acid data, with a heating temperature of 0.008. Convergence was achieved as the standard deviation of split frequencies for each dataset was below 0.01, and the initial 20% of trees were discarded as burn-in. The consensus tree (with posterior probabilities; BPP) was visualised and edited in FigTree v. 1.4.2 (Rambaut, 2014). Nodes with BPP of ≥ 0.95 were considered as strongly supported. For the Parsimony analyses (PAUP* 4.0b2.; Swofford, 2003), tree searches were performed using starting trees obtained via stepwise addition, with simple addition of taxon and tree-bisection-reconnection (TBR) branch swapping under the heuristic tree search option. The robustness of nodes was assessed through 1 000 bootstrap replications (Felsenstein, 1985) and were regarded as supported when the value was ≥ 75%.

*Estimating dates of evolutionary divergence*

In the absence of Collembola fossil calibration points, a generalised molecular clock (i.e., projected rates of genetic mutations) was used to calculate the estimates of divergence times between *Seira* lineages. Based on previous studies comparing geological and molecular data, mutation rates between 1.5% and 2.3% per million years (myr) are regularly applied to

arthropod COI nucleotide data (Brower, 1994; Gaunt & Miles, 2002; Quek et al., 2004). These rates were recently verified for a large group of arthropods using a lineage specific approach (see e.g. Mortimer et al., 2011). The mean arthropod rate of $1.9 \times 10^{-8}$ per myr was employed in the present study.

The nucleotide dataset was analysed employing the Bayesian framework in BEAST v. 1.8.0. (Drummond et al., 2012). It used the best-fitted evolutionary model together with a Yule speciation model (Heled & Drummond, 2011) and uncorrelated log-normal relaxed molecular clock (Drummond et al., 2006). The Bayesian relaxed-clock model allows the evolutionary rate to vary among lineages (Drummond et al., 2006). The monophyly of nodes with a posterior probability ≥ 0.95 was constrained. Analysis was re-run five times for 50 million generations sampling every 5 000$^{th}$ generation. This allowed the assessment of convergence in divergence time estimates. The sampling was considered adequate when the effective sample sizes (ESS) of posterior distributions and continuous parameters were greater than 200, as visualised using Tracer v. 1.6 (Rambaut & Drummond, 2013). The resulting trees of the five independent runs were combined using LogCombiner v. 1.8.0 (Drummond et al., 2012), and the maximum clade credibility consensus tree with mean node heights was constructed in TreeAnnotator v. 1.8.0 (Drummond et al., 2012) after the initial 20% were removed as burn-in. The age estimate of each node with 95% highest probability density (HPD) was obtained. FigTree v. 1.4.2 was used to visualise and edit the consensus tree (Rambaut, 2014).

## Results

*MOTU delineation*

Sequences of a 658 bp COI fragment were successfully obtained from 469 *Seira* specimens (331 collected from Fynbos and 138 from the Southern Afrotemperate forest). These individuals were previously grouped into 80 morphospecies by morphological characters prior to DNA sequencing. Sequence divergence values within morphospecies varied from 0% (all individuals were identical) to 28.40%, with some of the morphospecies not forming monophyletic clusters. Thus, some of these *a priori* identified morphospecies may in fact represent cryptic species. In an attempt to identify true evolutionary groups, both sequence divergence values (including barcoding gap analyses) as well as monophyletic clustering in the tree topologies were used to recognise distinct MOTUs. The NJ tree, constructed from uncorrected *p*-distances, is presented in Figure 2.2. A cursory examination of the tree indicated that terminal taxa converged within a short time period into groups, with comparatively longer branches separating these groups. The sequence divergence at which monophyletic groups, or MOTUs, are formed, was ≤ 2.50%. Based on this, a cut-off sequence divergence of 2.50% was proposed to delineate *Seira* MOTUs, which may correspond to species. This value was supported by the gap analyses (see Figure 2.3B), which showed a notable gap in the intra- and inter-MOTU divergences. This approach resulted in 91 MOTUs of which 20 were retrieved as singletons. The overall species richness increased by 13.75% from the morphologically defined species.

There were, however, some exceptions (i.e. with 16 MOTUs). For example, clades with grey blocks on the NJ tree (Figure 2.2) formed monophyletic groups, but each of these MOTUs had intra-MOTU divergence values above 2.50% and the highest was 8.10%. These were the clades responsible for the slight overlap between the ranges of intra- and inter-MOTU divergences in the barcoding gap analyses and removal of these clades resulted in a clear gap in the gap analyses; see Figure 2.3A and B. A detailed examination of these clades indicated large morphological similarity of the specimens grouping within them. Within these clades, member specimens were collected from the same geographical area of a particular habitat type (i.e. Fynbos or Southern Afrotemperate forest), or areas that were geographically closely situated of the same habitat type. In the absence of any distinguishing morphological features, as well as the presence of monophyletic clustering in the tree, these clades were considered as single MOTUs in the present study. However, further investigation into these clades is required to resolve their species status.

*Habitat assignment*

A few individuals from eight separate MOTUs were regarded as tourists to either Fynbos or Southern Afrotemperate forest habitat. In the majority of cases (six MOTUs), the tourists were found in Fynbos habitat while in two cases, the tourists were found in forest habitat (Grootvadersbosch and Baviaanskloof; see Table 2.1). Of the 91 MOTUs, 83 (91%) appeared to be habitat specialists. Sixty-three were unique to Fynbos habitat, and of these 55 appeared to have narrow distributions (i.e. present in only one locality) and only eight were found in areas more than 35 km apart. Twenty of the MOTUs were uniquely linked to the Southern Afrotemperate forest, and of these 18 had a restricted distribution. Only eight generalist MOTUs were identified based on occurrence in both habitat types.

*Phylogenetic analyses and estimating dates of evolutionary divergence*

Next I investigated whether *Seira* MOTUs predominantly found in a specific habitat type might consistently be older or younger than the MOTUs from other habitat types. For this, 206 specimens were selected as representatives of the larger dataset, with a minimum of two specimens per MOTU and only one specimen for the rare MOTUs. The three outgroup taxa (see Materials and Methods) were included to root evolutionary topologies. A consistent finding was that not all of the outgroup taxa clustered outside the ingroup. Specifically, the representatives of Entomobryidae and Isotomidae consistently grouped inside the ingroup with only the Hypogastruridae behaving like a true outgroup. This can be explained when considering the high level of sequence divergences separating taxa. The highest sequence divergence within the *Seira* group was 31.10%. Sequence divergence values to the three outgroup taxa were similar (see Table 2.2). These results should not be interpreted as questioning the higher-level taxonomy of Collembola, but rather that the COI gene evolves too rapidly to be used to reliably distinguish relationships between distantly diverged taxa.

Given the high sequence divergences separating taxa, analyses were performed for both nucleotide and amino acid datasets (Breinholt & Kawahara, 2013; Liu et al., 2014). For the Bayesian Inference topologies, a general time reversible (GTR) model with a proportion of invariable sites (= 0.51) and a gamma shape distribution (= 0.44) was selected as the most appropriate nucleotide substitution model based on the AIC criteria; no correction was done for the amino acid data as divergences were low. Although there were differences in the deeper relationships and nodal supports between the nucleotide and amino acid phylogenies (this is to

be expected given the saturation present in the nucleotide data for deep divergences), the within-clade composition displayed only minor conflicts between the datasets. The nucleotide data resolved the more recent relationships with more support (Figures 2.4) while the amino acid sequences generated good resolution and BPP support for the deeper relationships (Figure 2.5).

The BI analysis of the nucleotide data retrieved 16 statistically well-supported (BPP ≥ 0.95) clades: seven (clade F, J – L, N – P) were comprised of Fynbos specimens only, two (clade I and M) were forest individuals only and seven (clade A – E, G and H) comprised of specimens collected in both habitat types (Figure 2.4). Overall, the *Seira* specimens of Fynbos and Southern Afrotemperate forest habitat did not form distinct monophyletic groups. In mixed habitat clades (A, E, G and H), forest specialists or habitat generalists were always basal to Fynbos individuals. There was no instance where Fynbos specialists were basal in the mixed habitat clades. Clade A with a combination of both Fynbos and forest MOTUs formed a basal group to mixed habitat clade B and C. Forest specimens were basal to statistically well-supported clusters comprising of only Fynbos specimens in clade G and H. Using the amino acid sequences, one of the forest MOTUs from Grootvadersbosch was recovered as basal to the remaining MOTUs in the Bayesian phylogenetic tree with strong BPP support (Figure 2.5).

The divergence time suggested that the root date for the *Seira* complex is approximately 21.86 mya (24.82 – 18.72 mya; Late Oligocene to Early Miocene) (Figure 2.6). Within the CFR, the divergences between *Seira* MOTUs appear to have mainly occurred during the Miocene, while the more recent radiations occurred during the Pleistocene and Holocene within the MOTUs. The divergences between Fynbos and forest MOTUs seemed to cluster in a relatively narrow time, namely during the Mid-Miocene. Specifically, within clade G, the divergence of Grootvadersbosch forest specimens and the Western Cape Fynbos clusters occurred around 11.75 mya (14.37 – 8.94 mya) (Figure 2.6), while the divergence between Wilderness forest specimens and the Western-Eastern Cape Fynbos group dated to around 12.22 mya (clade H: 14.37 – 9.90 mya) (Figure 2.6). The divergence of some of the Table Mountain (Cape Peninsula) specimens from other areas in the Western Cape occurred between 11.13 and 3.84 mya (within clade B, E, I, J, O and P) (Figure 2.6). The forest specimens from Tsitsikamma diverged from the Outeniqua forest and Baviaanskloof Fynbos and forest individuals at 14.32 mya (clade A: 16.90 - 12.01 mya) (Figure 2.6). Similar dates of divergence occurred between Tsitsikamma forest individuals, and

Outeniqua and Grootvadersbosch forest specimens in clade M (12.37 mya; 15.29 – 9.35 mya) (Figure 2.6).

## Discussion

This is the first study that has investigated a soil mesofauna group, more specially the Collembola, in southern Africa using molecular markers. Previous studies of *Seira* included only taxonomic assessments based on morphological variations (Börner, 1908; Womersley, 1934; Paclt, 1959, 1967; Yosii, 1959; Coates, 1968; Bellinger et al., 2015). Several notable results emerged from this study, including an unexpectedly high number of MOTUs, as well as high levels of sequence divergences separating these. Substantial cladogenesis within the group seemed to occur in the Miocene with more recent events in the Pleistocene and Holocene. These findings are discussed in more detail below.

### *MOTU (species) diversity*

Previous studies on *Seira* in South Africa recognized 29 species (Börner, 1908; Womersley, 1934; Paclt, 1959, 1967; Yosii, 1959; Coates, 1968); with 21 of these known to occur in the Western Cape (Coates, 1968). Given that the MOTU richness in the Fynbos and forest regions of the CFR is notably high, it is likely that the previous alpha taxonomic studies of this genus have substantially underestimated the species richness. Based on a holistic approach (sequence divergence threshold of 2.5% with higher sequence divergence separating monophyletic groups compared with intra-MOTU divergences; confirmed by barcoding gap analyses; Hebert et al. 2004, Meyer & Pauley, 2005), no less than 91 MOTUs were delineated. This adds to a growing body of literature confirming that the true Collembola species richness is significantly underestimated (Deharveng, 2004). It also highlights the remarkable radiation and diversity of this genus in the CFR, which is uncommon in other arthropods with the exception of some plant specialist groups. Indeed, previous results have shown that the Greater Cape is the global and/or South African centre of diversity for several insect pollinator and plant-specialist groups, such as the lacewings (Sole et al., 2013), weevils (Colville et al., 2014) and Holpiini monkey beetle (Colville, 2009).

The high number of MOTUs (91 clades) is supported by our finding of a similar number of morphospecies (80). Moderately higher MOTU richness is common in inconspicuous soil living organisms for which differences in morphology may often be difficult to distinguish (see e.g. Diplura: Podsiadlowski et al., 2006; Torricelli et al., 2010; various Collembola groups: Frati et al., 2000; Timmermans et al., 2005; Emerson et al., 2011; Porco et al., 2012b, 2014; Cicconardi et al., 2013). Consequently, it has been proposed that traditional morphological characters may

sometimes be too conservative to reflect the extensive genetic differentiation detected in Collembola (Soto-Adames, 2002). Deeply divergent evolutionary lineages (based on mitochondrial and nuclear markers) are frequently found within morphologically recognised Collembola species (Garrick et al., 2004, 2007; Timmermans et al., 2005; Cicconardi et al., 2010; 2013; Torricelli et al., 2010).

The morphological conservatism over evolutionary time reported in many Collembola morphospecies (Emerson et al., 2011; Katz et al., 2015) could be due to strong environmental constraints (Torricelli et al., 2010) or convergence. The soil environment may have acted as a buffer, potentially slowing the morphological differentiation in certain Collembola species (Cicconardi et al., 2010). Moreover, morphological characters such as scales and colour/pigmentation pattern may sometimes be unreliable characters due to within-species variability, juveniles and/or the presence of sibling species (Frati et al., 2000). In the present study, although there was some discordance between morphospecies and molecular species units, differences in body pigmentation patterns appeared to be an important morphological character in discriminating some of the *Seira* species (see Supplementary Figure S1), although colour is not always useful given the direct exposure to ethanol (see also Coates, 1968). The importance of pigmentation patterns in discriminating other Collembola species has been previously shown by Soto-Adames (2002).

*Habitat assignment*

Overall, most of the *Seira* MOTUs appeared to be both geographically localised and habitat specific. It has been suggested that Collembola are capable of long distance passive dispersal mediated by wind (Freeman, 1952; Farrow & Greenslade, 1992; Dunger et al., 2002), larger animals or humans. However, these events may be rare in *Seira* of the CFR, as the results of this study support ecological structuring in the majority of the *Seira* assemblages in the region, evidenced by only a few of the MOTUs occurring in both Fynbos and Southern Afrotemperate forest habitats. This division between habitats and their soil occupants could be due to the dissimilarity in environmental conditions (i.e. temperature and humidity) and resource availability between the habitat types (Cowling & Holmes, 1992; Manders et al., 1992; Manders & Richardson, 1992). Furthermore, habitat specialists typically have a narrow range of local environmental conditions in which they can survive.

In a few instances, higher intra-MOTU divergences (above 2.5%) were displayed in clades (Figure 2.2). These MOTUs (which cluster as monophyletic groups, distinct from other groups) invariably comprised habitat generalists (sampled in both the Fynbos and forest habitats), with member individuals sampled across large geographic ranges (separated by > 50 km). A closer examination of these larger MOTUs does reveal some structure (sampling localities tend to form smaller clusters within the larger MOTU), but the separation is not clear enough to warrant individual recognition. Further studies should be done to understand whether incipient speciation is occurring, or whether connectivity between sampling areas is high.

The *Seira* MOTU richness recorded in the Fynbos habitat was higher than forest habitats. The contemporary *Seira* MOTU richness in Fynbos may be related to the high diversification rate and relatively low rates of extinction in the Cape flora lineages (Dynesius & Jansson, 2000; Midgley et al., 2001; Cowling & Lombard, 2002; Latimer et al., 2005), whereas the diversity of forest vegetation had been declining dramatically since the mid Miocene (Linder et al., 1992; Goldblatt & Manning, 2002). Therefore, the *Seira* lineages associated with the Fynbos habitat may have greater opportunities for radiations than that of their counterparts from the forest habitats.

*Divergence times*

It is important to emphasise that there are considerable uncertainties about the reconstruction of divergence dates for *Seira* based on only a single mitochondrial gene, thus the results presented here are highly speculative. It was found that many of the deeper diversifications of the Fynbos and forest *Seira* lineages occurred during the Miocene, which also appears to have been an important period for the diversification of other invertebrate taxa in South Africa (Daniels et al., 2009, 2013). The Early Miocene in the CFR was characterised by the initiation of the mild Post-African I uplift (Partridge, 1997b), which increased the topographic and edaphic heterogeneity of both lowland and mountainous areas in the region (Cowling et al., 2009). It is possible that some of the ancestral lineages of Fynbos-inhabiting *Seira* specimens existed prior to the Miocene, but they would potentially have been restricted to the oligotrophic sandstone mountains, as was suggested for the ancestral Cape flora lineages (Linder, 2003; Verboom et al., 2009). The Mid to Late Miocene was marked by major changes in regional climatic regimes, and the summer arid climate associated with the Benguela upwelling system became established by the Late Miocene

(Siesser, 1980; Diekmann et al., 2003; Linder, 2003; Krammer et al., 2006). The widespread recurrent fire regime may also have been established in the Mid Miocene (Bytebier et al., 2011). Furthermore, the major Post-African II uplift initiated in the Late Miocene caused considerable tectonic uplifts in the region (Cowling et al., 2009). Subsequently, these pronounced palaeoclimatic changes, tectonic activities and natural recurrent fire systems are thought to have triggered the fragmentation and decline in diversity of forest elements, as well as the increased expansion and radiation of the more xeric and fire-prone shrubland vegetation (Linder et al., 1992; Geldenhuys, 1997; Dupont et al., 2011), which may affect the diversification of associated *Seira* taxa.

Interestingly, sister taxon relationships exist between taxa inhabiting the Southern Afrotemperate forest occurring in various localities (i.e. Tsitsikamma, Outeniqua and Baviaanskloof/Tsitsitkamma, Outeniqua and Grootvadersbosch/Table Mountain and other Western Cape forests). This may suggest that *Seira* were once continuously distributed, and that forests were more extensive and continuous prior to the onset of Mid Miocene climatic changes. The historical connection between the Cape Peninsula forest and the south-western Cape forests has also been proposed for the velvet worms (Daniels et al., 2009). The divergence between forest-inhabiting and Fynbos *Seira* clusters of the two clades in the Mid Miocene also corresponded with the approximate timing of transitions in vegetation structure within the CFR, where Fynbos-inhabiting *Seira* radiated into numerous Fynbos habitats across the region. The general presence of forest *Seira* at the basal position of the mixed habitat clades or Fynbos clusters could be explained by the predominance of forest elements in the Palaeogene flora fossils, as it dominated most of Africa before the Late Miocene epoch (Dupont et al., 2011; Roberts et al., 2011; Verboom et al., 2014). It seems that some of the forest *Seira* lineages may have shifted to the Fynbos habitats and subsequently radiated in the Fynbos with some of the lineages later relocating back into the forests.

Some of the *Seira* species of Table Mountain appeared to show divergence from the adjacent sampled areas in the Western Cape, suggesting isolation of the Cape Peninsula *Seira* from the other mountain blocks (Boland, Hottentots-Holland, Overberg and Langeberg) by the low-lying Cape Flats. This coincided with the estimated timing of phylogeographic breaks in other invertebrates (Gouws et al., 2004; McDonald & Daniels, 2012; Daniels et al., 2013). The divergences were likely a response to the climatic oscillations and the geomorphic change with the glacial-interglacial cycles throughout the Pliocene and Quaternary in the region (Cowling et

al., 2009). The diversification of *Seira* appeared to have remained high throughout the Quaternary with mild climatic disturbances, which may have further contributed to the contemporary distribution and richness of *Seira* biota in the region. Several other invertebrate studies also suggest taxon diversification in Quaternary (Price et al., 2007, 2010; Daniels et al., 2013; Sole & Scholtz, 2013).

Overall, the complex environmental conditions may have led to the general occurrences of geographically localised and ecologically specialised *Seira* MOTUs in the region, with few widespread generalists. The high degrees of specialisation and potentially low mobility are likely to have facilitated long-term isolation and speciation. The deep sequence divergences found among the MOTUs imply that they have not experienced genetic exchange or shared the same evolutionary history for a long time. Other Collembola investigated to date showed similar findings of geographically distinct and geologically old molecular lineages (Stevens & Hogg, 2003; Garrick et al., 2007; Cicconardi et al., 2010, 2013; Torricelli et al., 2010).

*Conclusions*

The results found in this study provide the necessary first interpretations and perspectives into the species discovery and evolutionary relationships of this understudied Collembola genus in the CFR. Here, the mitochondrial COI barcoding data support previous studies demonstrating the effectiveness of the COI gene in species-level discovery and the unmasking of cryptic taxa which were previously unrecognised by morphological variation alone (Stevens & Hogg, 2003; Hogg & Hebert, 2004).

For future work, phylogenetic analysis should be complemented with multiple loci to fully infer genealogical relationships and increase the phylogenetic resolution. Although this study suggests that the number of species in the genus *Seira* in the CFR may be four times as diverse as suggested by present taxonomic studies, even this is likely to be a significant under-estimation. Although most of the larger forest patches in the region were sampled, several smaller forest patches and a large fraction of the available Fynbos remain to be included in the investigation. Furthermore, other biomes should also be included in future studies to maximise species discovery, as it is plausible that there are many more undocumented species in unsampled habitat types. Given the high degrees of habitat preference and small distribution ranges suggested by many of these *Seira* MOTUs, it is likely that this represents only a fraction of the Collembola

richness in the CFR. This study also highlights the need for detailed revision of the genus *Seira* occurring in the CFR. An approach using a combination of traditional morphology, molecular data, and other lines of evidence such as ecological preferences and physiological constraints will provide a holistic approach to disentangle species groups and assessing the species boundaries of *Seira*.

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**Table 2.1.** Specimens of the genus *Seira* collected from the Western and Eastern Cape Province of South Africa. *N* indicates the total number of specimens sequenced per MOTU. *N** indicates the total number of specimens used in the phylogenetic analyses for each MOTU. NA denotes instances where information is not available. Bold indicates MOTUs with tourist individuals. The MOTU codes correspond to Figure 2.2, 2.4 – 2.6. NR – Nature Reserve; NP – National Park; WA – Wilderness Area; FR – Forest Reserve; BG – Botanical Garden.

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| Ingroup | | | | | | | | | |
| 1 | WC | Mount Hebron NR, Kogelberg | -34.28175 | 19.11230 | 348 | 10 | 3 | Fynbos, beating/aspirating | W.P.A. Liu |
| | WC | Kogelberg NR | -34.32935 | 18.95913 | NA | | | Fynbos, ant nest | C. Janion |
| 2 | EC | Toarwater, Baviaanskloof | -33.66132 | 23.77098 | 1274 | 4 | 3 | Fynbos, beating/aspirating | W.P.A. Liu |
| | EC | Holgat Pass, Baviaanskloof | -33.63683 | 24.45168 | 529 | | | Fynbos litter | W.P.A. Liu |
| | EC | Kouga WA, Baviaanskloof | -33.71171 | 23.83633 | 611 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| 3 | WC | Grootvadersbosch NR, Langeberg | -33.97464 | 20.83545 | 470 | 6 | 2 | Fynbos litter | W.P.A. Liu |
| 4 | EC | Kouga WA, Baviaanskloof | -33.71094 | 23.83507 | 577 | 4 | 2 | Fynbos litter | W.P.A. Liu |
| 5 | EC | Holgat Pass, Baviaanskloof | -33.63681 | 24.45136 | 528 | 6 | 2 | Fynbos, beating/aspirating | C. Janion/W.P.A. Liu |
| 6 | WC | De Hoop NR, Potberg, Overberg | -34.40550 | 20.56498 | 121 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| 7 | WC | De Hoop NR, Potberg, Overberg | -34.41279 | 20.59224 | 141 | 4 | 2 | Fynbos litter | W.P.A. Liu |
| 8 | WC | Silvermine NR, Table Mountain | -34.08677 | 18.41927 | 316 | 17 | 5 | Fynbos litter, beating/aspirating | W.P.A. Liu |
| | WC | Tokai FR, Table Mountain | -34.0536 | 18.43565 | NA | | | Fynbos litter | C. Janion |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| 8 | WC | Helderberg NR | -34.05849 | 18.87574 | 170 | 17 | 5 | Fynbos litter | W.P.A. Liu |
| | WC | Vergelegen Estate, Helderberg | -34.07713 | 18.93697 | 329 | | | Fynbos litter | W.P.A. Liu |
| | WC | Harold Porter BG, Kogelberg | -34.34770 | 18.92717 | 72 | | | Fynbos litter | W.P.A. Liu |
| | WC | Houwhoek NR, Kogelberg | -34.20937 | 19.15604 | 289 | | | Fynbos litter | W.P.A. Liu |
| | WC | Kogelberg NR | -34.30238 | 18.94169 | 61 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| | WC | De Hoop NR, Overberg | -34.43832 | 20.41900 | 47 | | | Fynbos litter | W.P.A. Liu |
| 9 | WC | De Hoop NR, Overberg | -34.43817 | 20.41940 | 43 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| 10 | EC | Kouga WA, Baviaanskloof | -33.71153 | 23.83602 | 604 | 2 | 2 | Fynbos litter | W.P.A. Liu |
| | EC | Koos se Bos, Baviaanskloof | -33.70459 | 23.82993 | 560 | | | Forest litter | W.P.A. Liu |
| 11 | EC | Otter trail, Tsitsikamma NP | -33.97498 | 23.62700 | 193 | 4 | 2 | Fynbos litter | W.P.A. Liu |
| | WC | Nature's Valley, Tsitsikamma NP | -33.96861 | 23.53712 | 218 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| 12 | WC | Outenqiua Pass, Outeniqua NR | -33.91342 | 22.41102 | 506 | 5 | 2 | Fynbos litter | W.P.A. Liu |
| | WC | Montagu Pass, Outeniqua NR | -33.93376 | 22.42522 | 253 | | | Forest litter | W.P.A. Liu |
| 13 | WC | Montagu Pass, Outeniqua NR | -33.88587 | 22.42190 | 538 | 3 | 2 | Forest litter | W.P.A. Liu |
| 14 | WC | Montagu Pass, Outeniqua NR | -33.88770 | 22.42412 | 512 | 4 | 2 | Forest litter | W.P.A. Liu |
| 15 | WC | Montagu Pass, Outeniqua NR | -33.93257 | 22.42452 | 239 | 1 | 1 | Forest litter | W.P.A. Liu |
| 16 | WC | Montagu Pass, Outeniqua NR | -33.90087 | 22.42035 | 537 | 8 | 3 | Fynbos litter | W.P.A. Liu |
| | WC | Nature's Valley, Tsitsikamma NP | -33.97007 | 23.60581 | 231 | | | Fynbos litter | W.P.A. Liu |
| | EC | Otter trail, Tsitsikamma NP | -33.97498 | 23.62700 | 193 | | | Fynbos litter | W.P.A. Liu |
| 17 | WC | Montagu Pass, Outeniqua NR | -33.87397 | 22.44753 | NA | 2 | 2 | Fynbos litter | W.P.A. Liu |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| 17 | WC | Outeniqua Pass, Outeniqua NR | -33.90586 | 22.40538 | 637 | 2 | 2 | Fynbos litter | W.P.A. Liu |
| 18 | WC | De Hoop NR, Overberg | -34.41297 | 20.59286 | 145 | 9 | 4 | Fynbos litter | W.P.A. Liu |
| | WC | De Hoop NR, Potberg, Overberg | -34.44188 | 20.42017 | 38 | | | Fynbos litter | W.P.A. Liu |
| | WC | Grootvadersbosch NR, Langeberg | -33.97665 | 20.81602 | 507 | | | Fynbos litter | W.P.A. Liu |
| | WC | Montagu Pass, Outeniqua NR | -33.87208 | 22.44977 | NA | | | Fynbos litter | W.P.A. Liu |
| 19 | WC | Silvermine NR, Table Mountain | -34.09531 | 18.42489 | 277 | 76 | 11 | Fynbos litter, beating/aspirating | W.P.A. Liu |
| | WC | Jonkershoek NR | -33.99598 | 18.95260 | NA | | | Fynbos, beating/aspirating | L. Deharveng/A. Bedos |
| | WC | Helderberg NR | -34.0635 | 18.87186 | 72 | | | Fynbos litter, beating/aspirating | W.P.A. Liu |
| | WC | Harold Porter BG, Kogelberg | -34.34508 | 18.92705 | 99 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| | WC | Kogelberg NR | -34.30790 | 18.94500 | 52 | | | Fynbos litter, beating/aspirating | W.P.A. Liu |
| | WC | De Hoop NR, Overberg | -34.37372 | 20.54560 | NA | | | Fynbos, beating and aspirating | C. Janion/W.P.A. Liu |
| | WC | Grootvadersbosch NR, Langeberg | -33.97672 | 20.81578 | 507 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| | WC | Montagu Pass, Outeniqua NR | -33.88108 | 22.43895 | NA | | | Fynbos, beating/aspirating | W.P.A. Liu |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| 19 | WC | Wilderness NP | -33.97650 | 22.68990 | 100 | 76 | 11 | Fynbos litter, beating/aspirating | L. Deharveng/A. Bedos |
| | EC | Otter trail, Tsitsikamma NP | -33.99653 | 23.74097 | 97 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| 20 | WC | Montagu Pass, Outeniqua NR | -33.88157 | 22.44050 | NA | 3 | 2 | Fynbos, beating/aspirating | W.P.A. Liu |
| | EC | Nature's Valley, Tsitsikamma NP | -33.97035 | 23.53704 | 220 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| 21 | WC | Montagu Pass, Outeniqua NR | -33.88053 | 22.42783 | 789 | 4 | 3 | Fynbos, beating/aspirating | W.P.A. Liu |
| 22 | WC | Silvermine NR, Table Mountain | -34.09578 | 18.42536 | 274 | 2 | 2 | Fynbos litter, beating/aspirating | W.P.A. Liu |
| 23 | WC | Silvermine NR, Table Mountain | -34.08672 | 18.41949 | 314 | 2 | 2 | Fynbos, beating/aspirating | W.P.A. Liu |
| | WC | Kogelberg NR | -34.30808 | 18.94465 | 62 | | | Fynbos litter | W.P.A. Liu |
| **24** | WC | Kogelberg NR | -34.33372 | 18.94889 | 238 | 20 | 6 | Forest litter | C. Janion/W.P.A. Liu/L.Deharveng/ A. Bedos |
| | WC | Mount Hebron NR, Kogelberg | -34.28187 | 19.11212 | 344 | | | Fynbos litter | W.P.A. Liu |
| | WC | Helderberg NR | -34.0413 | 18.87367 | 579 | | | Forest litter | L.Deharveng/W. P.A. Liu |
| | WC | Lourensford Estate, Helderberg | -34.0078 | 18.93822 | 649 | | | Forest litter | W.P.A. Liu |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| 25 | WC | Grootvadersbosch NR, Langeberg | -33.98314 | 20.82481 | 389 | 2 | 2 | Forest litter | W.P.A. Liu |
| 26 | WC | Grootvadersbosch NR, Langeberg | -33.97000 | 20.81778 | 632 | 4 | 2 | Fynbos, beating/aspirating | W.P.A. Liu |
| 27 | WC | Helderberg NR | -34.05614 | 18.87599 | 193 | 9 | 4 | Fynbos litter | W.P.A. Liu |
| | WC | Jonkershoek NR | -33.98990 | 18.95546 | NA | | | Fynbos litter | C. Janion |
| | WC | Houwhoek NR, Kogelberg | -34.21185 | 19.15850 | 293 | | | Fynbos litter | W.P.A. Liu |
| 28 | WC | Vergelegen Estate, Helderberg | -34.07647 | 18.92378 | 232 | 3 | 2 | Fynbos litter | W.P.A. Liu |
| 29 | WC | Helderberg NR | -34.05903 | 18.87588 | 163 | 6 | 5 | Fynbos litter | W.P.A. Liu |
| | WC | Vergelegen Estate, Helderberg | -34.07712 | 18.93719 | 338 | | | Fynbos litter | W.P.A. Liu |
| | WC | Harold Porter BG, Kogelberg | -34.34721 | 18.92776 | 65 | | | Fynbos litter | W.P.A. Liu |
| 30 | WC | Groot Wintershoek NR | -33.00723 | 19.05536 | 1002 | 3 | 2 | Fynbos litter | W.P.A. Liu |
| 31 | EC | Kouga WA, Baviaanskloof | -33.71166 | 23.83648 | 613 | 2 | 1 | Fynbos litter | W.P.A. Liu |
| 32 | WC | Groot Wintershoek NR | -33.0052 | 19.05537 | 991 | 1 | 1 | Fynbos, beating/aspirating | W.P.A. Liu |
| **33** | WC | Silvermine NR, Table Mountain | -34.0851 | 18.41746 | 332 | 21 | 7 | Fynbos litter | W.P.A. Liu |
| | WC | Inchuk Cave entrance, Table Mountain | -33.97770 | 18.40080 | NA | | | Forest litter | L. Deharveng/A. Bedos |
| | WC | Spes Bona, Table Mountain | -34.11539 | 18.44191 | 420 | | | Forest litter | W.P.A. Liu |
| | WC | Kirstenbosch BG, Table Mountain | -33.99039 | 18.43209 | NA | | | Forest litter | C. Janion/W.P.A. Liu |
| 34 | WC | Helderberg NR | -34.05870 | 18.87580 | 167 | 5 | 2 | Fynbos litter, beating/aspirating | W.P.A. Liu |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| 34 | WC | Vergelegen Estate, Helderberg | -34.07789 | 18.94011 | 341 | 5 | 2 | Fynbos litter | W.P.A. Liu |
| 35 | WC | Silvermine NR, Table Mountain | -34.08681 | 18.41906 | 317 | 4 | 2 | Fynbos litter | W.P.A. Liu |
| 36 | EC | Nature's Valley, Tsitsikamma NP | -33.96828 | 23.53718 | 220 | 4 | 2 | Fynbos, beating/aspirating | W.P.A. Liu |
| | WC | Otter trail, Tsitsikamma NP | -33.99662 | 23.74165 | 105 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| **37** | WC | Otter trail, Tsitsikamma NP | -33.97659 | 23.63189 | 221 | 6 | 3 | Fynbos litter | W.P.A. Liu |
| | EC | Stormriver, Tsitsikamma NP | -33.9658 | 23.86549 | 254 | | | Forest litter | W.P.A. Liu |
| 38 | EC | Stormriver, Tsitsikamma NP | -33.9658 | 23.86549 | 254 | 4 | 2 | Forest litter | W.P.A. Liu |
| 39 | EC | Nature's Valley, Tsitsikamma NP | -33.97243 | 23.60808 | 206 | 1 | 1 | Fynbos, beating/aspirating | W.P.A. Liu |
| 40 | EC | Kouga WA, Baviaanskloof | -33.71094 | 23.83507 | 577 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| **41** | WC | Grootvadersbosch NR, Langeberg | -33.97373 | 20.81757 | 545 | 10 | 2 | Fynbos litter | W.P.A. Liu |
| | WC | Grootvadersbosch NR, Langeberg | -33.98240 | 20.83457 | NA | | | Forest litter | W.P.A. Liu |
| 42 | WC | Grootvadersbosch NR, Langeberg | -33.98273 | 20.80873 | NA | 10 | 4 | Forest litter | C. Janion/W.P.A. Liu |
| | WC | Grootvadersbosch NR, Langeberg | -33.97665 | 20.81602 | 507 | | | Fynbos litter | W.P.A. Liu |
| 43 | WC | Grootvadersbosch NR, Langeberg | -33.97377 | 20.81734 | 542 | 4 | 2 | Fynbos litter | W.P.A. Liu |
| | WC | Grootvadersbosch NR, Langeberg | -33.98151 | 20.83653 | 278 | | | Forest litter | W.P.A. Liu |
| **44** | WC | Helderberg NR | -34.04183 | 18.87403 | 546 | 4 | 1 | Forest litter | W.P.A. Liu |
| | WC | Lourensford Estate, Helderberg | -34.00778 | 18.93822 | 649 | | | Forest litter | W.P.A. Liu |
| | WC | Kogelberg NR | -34.28725 | 18.88295 | 268 | | | Forest litter | W.P.A. Liu |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| **44** | WC | Grootvadersbosch NR, Langeberg | -33.98385 | 20.82526 | 369 | 4 | 1 | Forest litter | W.P.A. Liu |
| | WC | Grootvadersbosch NR, Langeberg | -33.98385 | 20.82526 | 369 | | | Fynbos litter | W.P.A. Liu |
| 45 | WC | Grootvadersbosch NR, Langeberg | -33.97205 | 20.82005 | 584 | 6 | 2 | Fynbos litter | W.P.A. Liu |
| | WC | Grootvadersbosch NR, Langeberg | -33.98310 | 20.82470 | 380 | | | Forest litter | W.P.A. Liu |
| **46** | WC | Susis trail, Jonkershoek NR | -33.99598 | 18.95256 | NA | 5 | 4 | Forest litter | L. Deharveng/A. Bedos |
| | WC | Helderberg NR | -34.04183 | 18.87403 | 546 | | | Forest litter | W.P.A. Liu |
| | WC | Mount Hebron NR, Kogelberg | -34.28200 | 19.11206 | 345 | | | Fynbos litter | W.P.A. Liu |
| | WC | Kogelberg NR | -34.28725 | 18.88295 | 268 | | | Forest litter | W.P.A. Liu |
| 47 | EC | Koos se Bos, Baviaanskloof | -33.70409 | 23.82947 | NA | 12 | 3 | Forest litter | C. Janion/W.P.A. Liu |
| | EC | Kouga, Baviaanskloof | -33.70409 | 23.82947 | NA | | | Forest litter | C. Janion |
| 48 | EC | Toarwater, Baviaanskloof | -33.66357 | 23.77337 | 1238 | 3 | 2 | Fynbos litter | W.P.A. Liu |
| | EC | Otter trail, Tsitsikamma NP | -33.97500 | 23.62725 | 200 | | | Fynbos litter | W.P.A. Liu |
| 49 | EC | Kestella se Bos, Baviaanskloof | -33.73320 | 23.96437 | 498 | 3 | 3 | Forest litter | W.P.A. Liu |
| | EC | Koos se Bos, Baviaanskloof | -33.70409 | 23.82947 | NA | | | Forest litter | C. Janion |
| | EC | Geelhoutbos, Baviaanskloof | -33.62850 | 24.24954 | 294 | | | Forest litter | W.P.A. Liu |
| 50 | EC | Toarwater, Baviaanskloof | -33.66096 | 23.77185 | 1279 | 5 | 2 | Fynbos litter | W.P.A. Liu |
| 51 | WC | Groot Wintershoek NR | -33.00723 | 19.05536 | 1002 | 1 | 1 | Fynbos, beating/aspirating | W.P.A. Liu |
| 52 | WC | De Hoop NR, Overberg | -34.40515 | 20.56518 | 121 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| 53 | WC | Cederberg NR | -32.46116 | 19.26268 | NA | 6 | 2 | Fynbos litter | C. Janion |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| 54 | WC | De Hoop NR, Overberg | -34.37372 | 20.54560 | NA | 6 | 2 | Fynbos litter | C. Janion |
| 55 | WC | Wilderness NR | -33.98065 | 22.65152 | NA | 8 | 3 | Forest litter | L. Deharveng/A. Bedos/C. Janion |
| 56 | WC | Krom river, Du Toitskloof | -33.71172 | 19.10932 | NA | 6 | 3 | Forest litter | D. Porco |
| 57 | WC | Top of Swartberg Pass, Oudtshoorn | -33.35433 | 22.04215 | 1673 | 6 | 2 | Fynbos, beating/aspirating | C. Janion |
| 58 | WC | Jonkershoek NR | -34.00560 | 18.99210 | NA | 6 | 2 | Fynbos litter | L. Deharveng/A. Bedos |
| 59 | WC | Tokai FR, Table Mountain | -34.05358 | 18.43565 | NA | 3 | 2 | Fynbos litter | C. Janion |
| 60 | WC | Montagu Pass, Outeniqua NR | -33.88725 | 22.42500 | 540 | 2 | 2 | Forest litter | W.P.A. Liu |
| 61 | WC | Outeniqua Pass, Outeniqua NR | -33.91357 | 22.41091 | 492 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| 62 | WC | Outeniqua Pass, Outeniqua NR | -33.91390 | 22.41060 | 504 | 4 | 4 | Fynbos, beating/aspirating | W.P.A. Liu |
| | EC | Nature's Valley, Tsitsikamma NP | -33.97017 | 23.53696 | 218 | | | Fynbos, beating/aspirating | W.P.A. Liu |
| 63 | EC | Geelhoutbos, Baviaanskloof | -33.62796 | 24.24987 | 303 | 2 | 2 | Forest litter | W.P.A. Liu |
| 64 | WC | Mount Hebron NR, Kogelberg | -34.2823 | 19.11164 | 337 | 2 | 2 | Fynbos litter | W.P.A. Liu |
| | WC | De Hoop NR, Potberg, Overberg | -34.4055 | 20.56498 | 121 | | | Fynbos litter | W.P.A. Liu |
| 65 | EC | Toarwater, Baviaanskloof | -33.66346 | 23.77173 | 1249 | 1 | 1 | Fynbos, beating/aspirating | W.P.A. Liu |
| 66 | EC | Toarwater, Baviaanskloof | -33.66132 | 23.77098 | 1274 | 1 | 1 | Fynbos litter | W.P.A. Liu |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| 67 | WC | Grootvadersbosch NR, Langeberg | -33.97390 | 20.82889 | 521 | 1 | 1 | Fynbos, beating/aspirating | W.P.A. Liu |
| 68 | WC | Grootvadersbosch NR, Langeberg | -33.97692 | 20.81536 | 501 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| **69** | WC | Grootvadersbosch NR, Langeberg | -33.97000 | 20.81778 | 632 | 4 | 3 | Fynbos litter, beating/aspirating | W.P.A. Liu |
| | WC | Grootvadersbosch NR, Langeberg | -33.98332 | 20.82910 | NA | | | Forest litter | W.P.A. Liu |
| 70 | WC | Grootvadersbosch NR, Langeberg | -33.97692 | 20.81536 | 501 | 4 | 3 | Fynbos litter | W.P.A. Liu |
| 71 | EC | Uitspan, Baviaanskloof | -33.50996 | 23.70203 | 856 | 2 | 2 | Forest litter | W.P.A. Liu |
| **72** | EC | Toarwater, Baviaanskloof | -33.66339 | 23.77149 | 1250 | 3 | 2 | Fynbos litter | W.P.A. Liu |
| | EC | Kestella se Bos, Baviaanskloof | -33.73384 | 23.96577 | 505 | | | Forest litter | W.P.A. Liu |
| 73 | WC | Grootvadersbosch NR, Langeberg | -33.98352 | 20.82952 | NA | 1 | 1 | Forest litter | W.P.A. Liu |
| 74 | WC | De Hoop NR | -34.42467 | 20.70523 | NA | 8 | 3 | Fynbos litter | C. Janion/W.P.A. Liu |
| 75 | WC | De Hoop NR, Potberg | -34.41300 | 20.59263 | 146 | 2 | 1 | Fynbos litter | W.P.A. Liu |
| 76 | WC | De Hoop NR, Potberg | -34.40560 | 20.56488 | 121 | 2 | 2 | Fynbos litter | W.P.A. Liu |
| 77 | WC | De Hoop NR | -34.43817 | 20.41940 | 43 | 2 | 2 | Fynbos litter | W.P.A. Liu |
| 78 | WC | Harold Porter BG, Kogelberg | -34.34508 | 18.92705 | 99 | 1 | 1 | Fynbos, beating/aspirating | W.P.A. Liu |
| 79 | WC | Harold Porter BG, Kogelberg | -34.34770 | 18.92717 | 72 | 2 | 2 | Fynbos litter | W.P.A. Liu |
| 80 | WC | Kogelberg NR | -34.33419 | 18.94720 | 276 | 1 | 1 | Forest litter | W.P.A. Liu |
| 81 | WC | Kogelberg NR | -34.30808 | 18.94465 | 62 | 2 | 2 | Fynbos litter | W.P.A. Liu |

**Table 2.1.** *continued*

| MOTU | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| 81 | WC | Houwhoek NR, Kogelberg | -34.20984 | 19.15564 | 293 | 2 | 2 | Fynbos litter | W.P.A. Liu |
| 82 | WC | Groot Wintershoek NR | -33.00723 | 19.05536 | 1002 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| 83 | WC | Silvermine NR, Table Mountain | -34.06668 | 18.38997 | 588 | 2 | 2 | Fynbos litter, beating/aspirating | W.P.A. Liu |
| 84 | WC | Silvermine NR, Table Mountain | -34.06676 | 18.39016 | 589 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| 85 | WC | Orangekloof NR, Table Mountain | -34.00412 | 18.39111 | 127 | 4 | 2 | Forest litter | W.P.A. Liu |
| | WC | Silvermine NR, Table Mountain | -34.08505 | 18.41746 | 333 | | | Fynbos litter | W.P.A. Liu |
| 86 | WC | Silvermine NR, Table Mountain | -34.06510 | 18.38745 | 615 | 4 | 2 | Fynbos litter | W.P.A. Liu |
| 87 | WC | Silvermine NR, Table Mountain | -34.07186 | 18.39760 | 482 | 22 | 4 | Fynbos litter, beating/aspirating | W.P.A. Liu |
| | WC | Orangekloof NR, Table Mountain | -33.99838 | 18.39237 | 155 | | | Forest litter, Fynbos beating/aspirating | C.Janion/W.P.A. Liu |
| | WC | Kirstenbosch BG, Table Mountain | -33.99039 | 18.43209 | NA | | | Forest litter | W.P.A. Liu |
| 88 | WC | Kogelberg NR | -34.30808 | 18.94465 | 62 | 2 | 2 | Fynbos litter | W.P.A. Liu |
| 89 | EC | Holgat Pass, Baviaanskloof | -33.63691 | 24.45184 | 528 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| 90 | WC | Houwhoek NR, Kogelberg | -34.20937 | 19.15604 | 289 | 1 | 1 | Fynbos litter | W.P.A. Liu |
| 91 | WC | De Hoop NR, Potberg | -34.41380 | 20.59760 | 139 | 3 | 1 | Fynbos litter | W.P.A. Liu |
| | | **Total number** | | | | **469** | **206** | | |

**Table 2.1.** *continued*

| Outgroup | Province | Site | Latitude | Longitude | Elevation (m) | *N* | *N** | Habitat/Sampling method | Collector/s |
|---|---|---|---|---|---|---|---|---|---|
| Entomobryidae | WC | Tokai FR, Table Mountain | -34.05460 | 18.43720 | NA | NA | 1 | Pine forest litter | C. Janion |
| Isotomidae | WC | White Water Lodge, Stanford | -34.40410 | 19.53850 | NA | NA | 1 | Fynbos litter | C. Janion |
| Hypogastruridae | WC | Boulders Beach, Marion Island | -46.87710 | 37.86080 | 5 | NA | 1 | *Sagina* leaves | C. Janion |

**Table 2.2.** Average sequence divergence of COI separating the ingroup from the outgroups. *For comparison, the highest sequence divergence among *Seira* MOTUs is given.

| | *Seira* MOTU | Hypogastruridae | Entomobryidae | Isotomidae |
|---|---|---|---|---|
| *Seira* MOTU | *31.100 | | | |
| Hypogastruridae | 24.752 | | | |
| Entomobryidae | 25.123 | 29.000 | | |
| Isotomidae | 24.978 | 26.500 | 24.600 | |

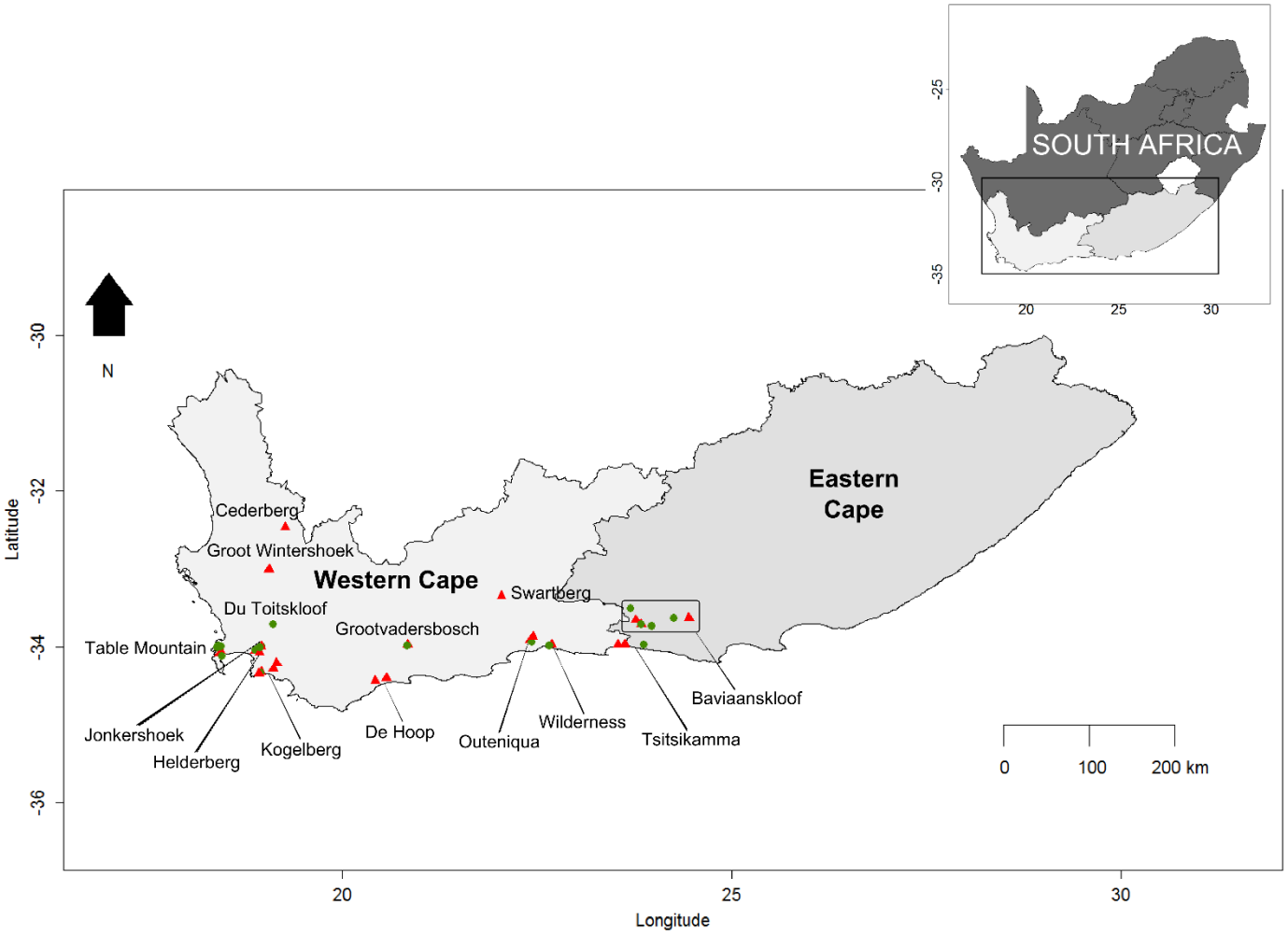
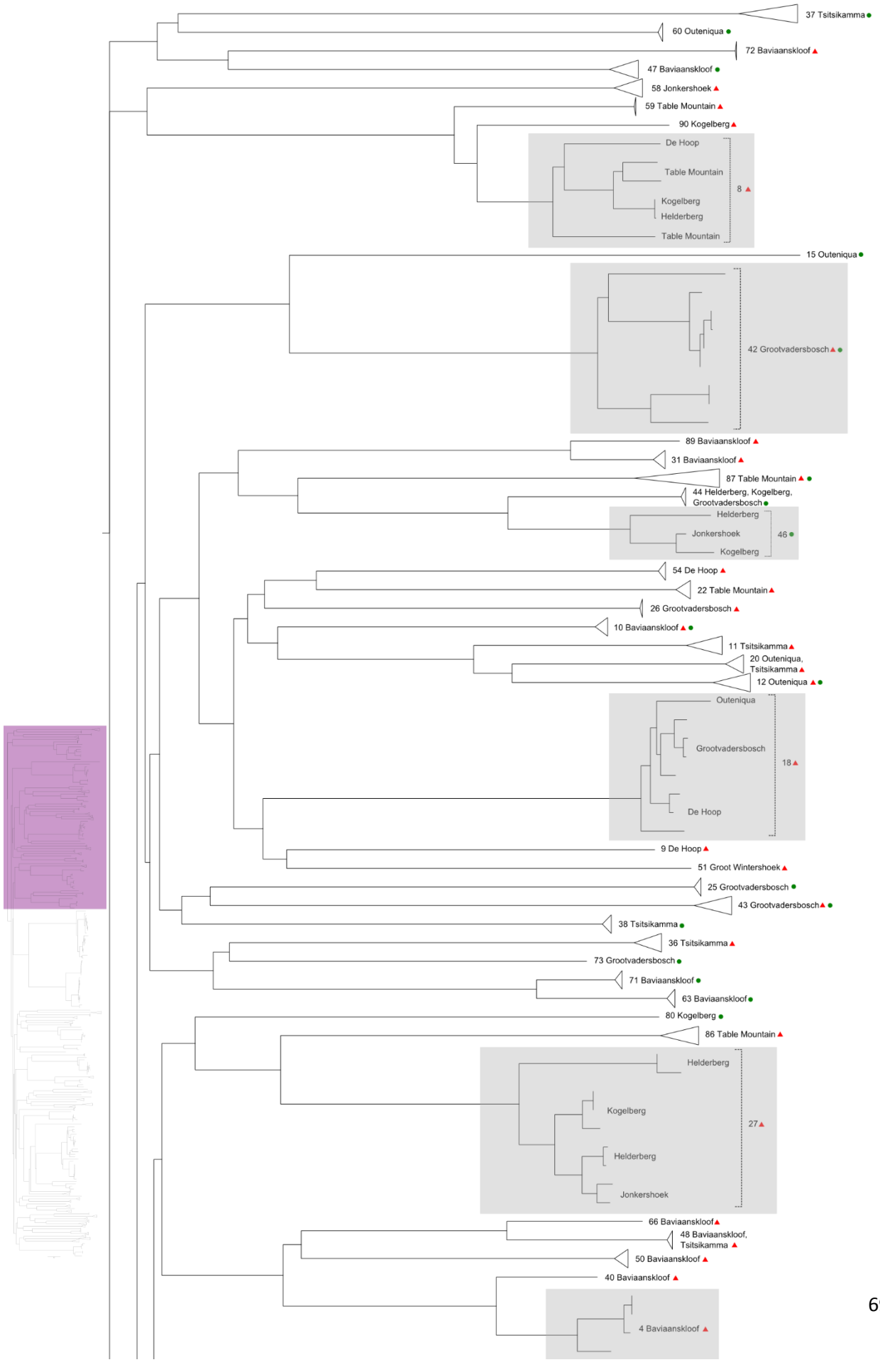
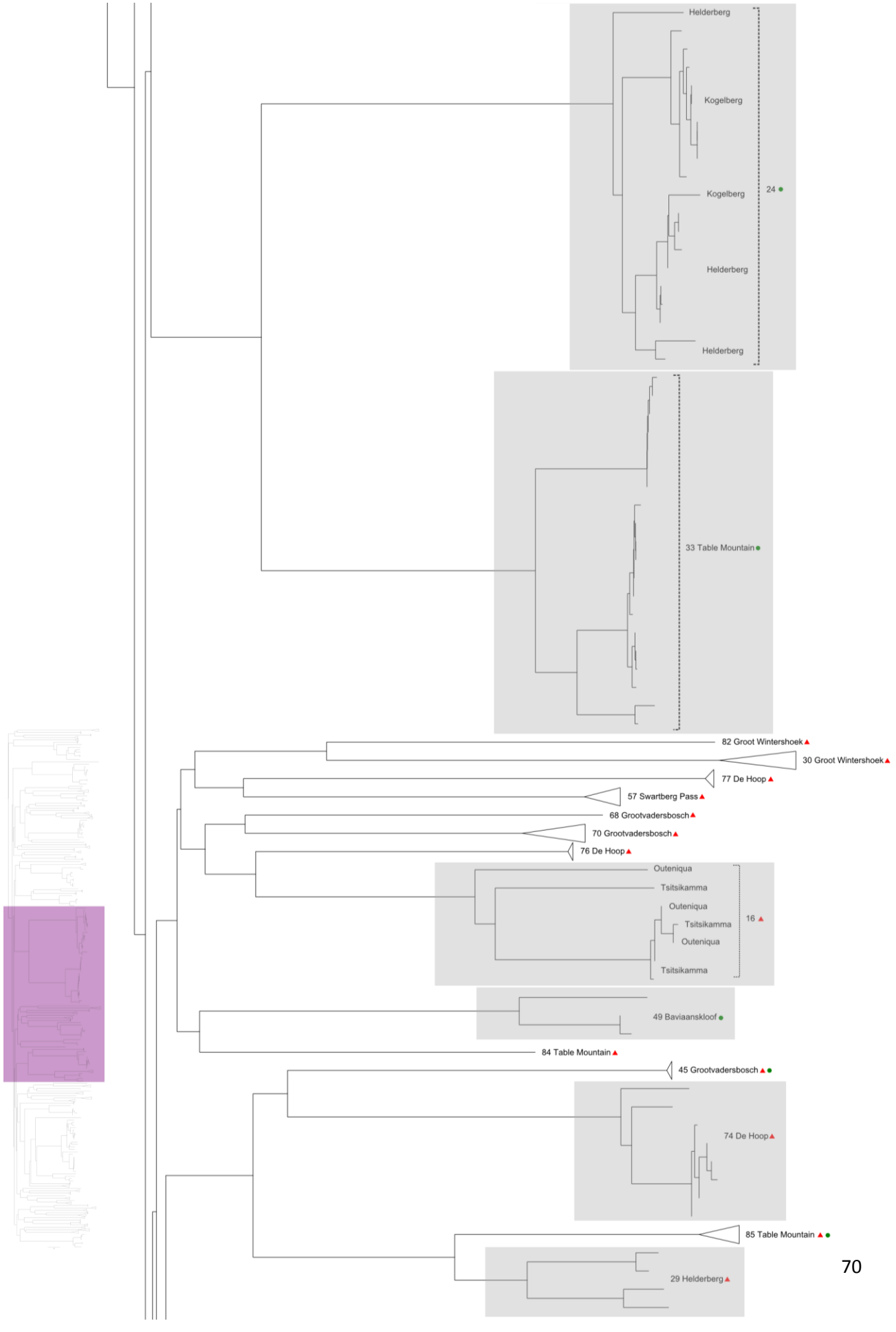



**Figure 2.1.** Locations of the 41 sampled sites, of which 23 were Fynbos (▲) and 18 were Southern Afrotemperate forest (●), in 14 areas of the Western and Eastern Cape Provinces of CFR.





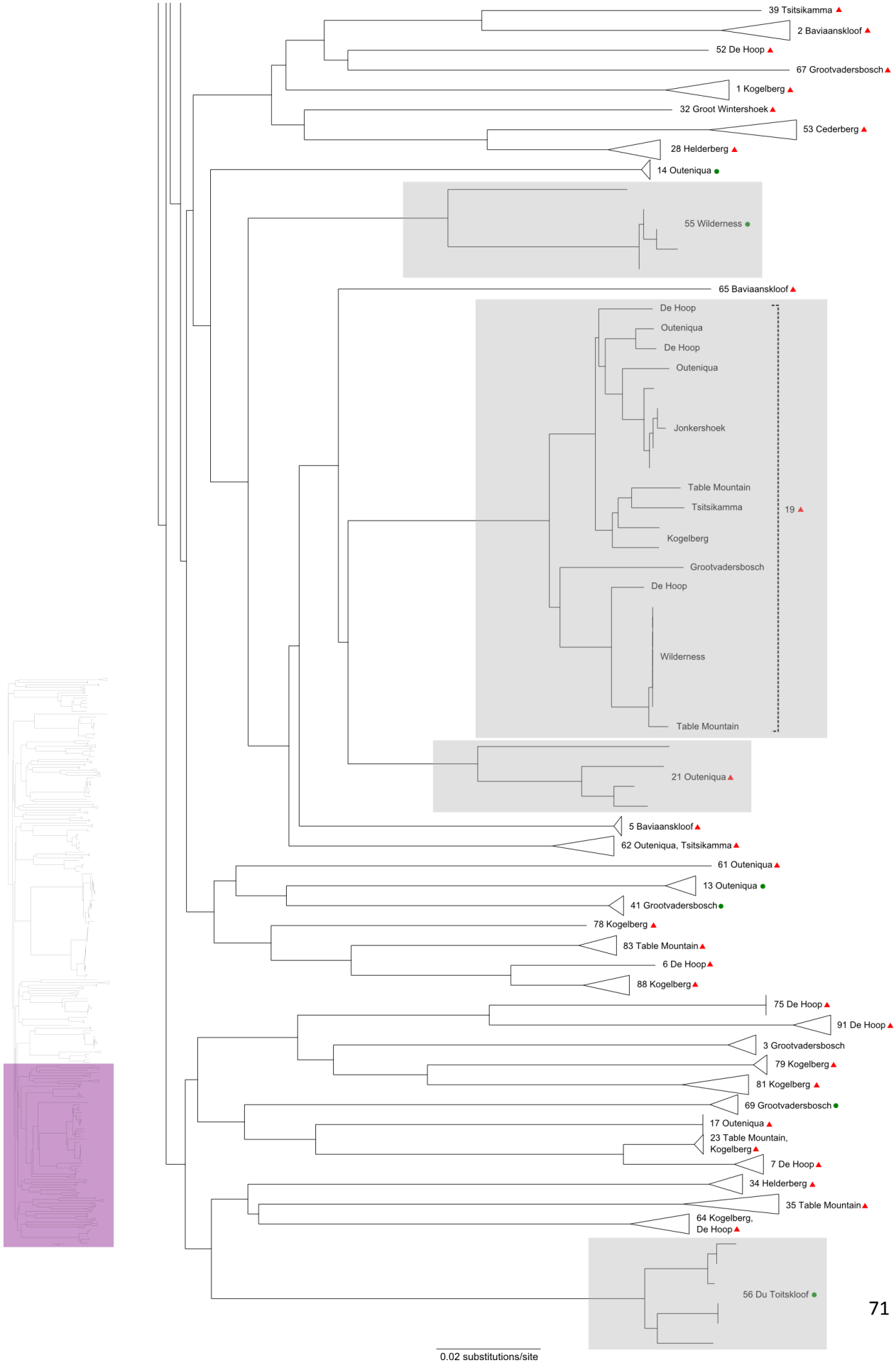


**Figure 2.2.** Neighbour-Joining tree based on uncorrelated *p*-distances for the 91 *Seira* MOTUs from the Fynbos (▲) and Southern Afrotemperate forest (●) using the COI nucleotide dataset. The 16 *Seira* MOTUs responsible for the slight overlap between the intra- and inter-MOTU divergences in the barcoding gap analyses are highlighted with grey blocks. MOTU codes correspond to Table 2.1. The section of the phylogeny shown on each page is highlighted in purple block on the left.

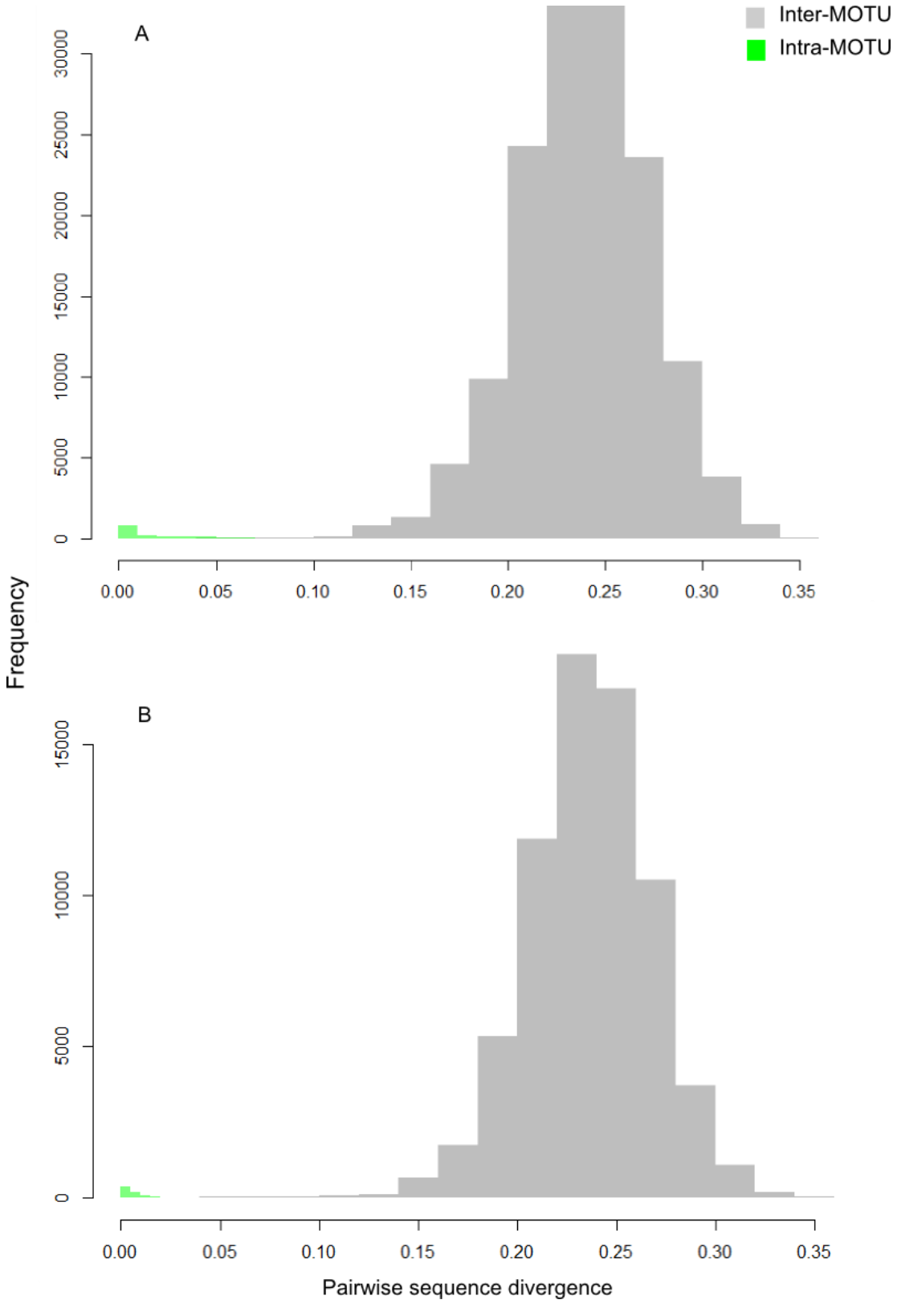
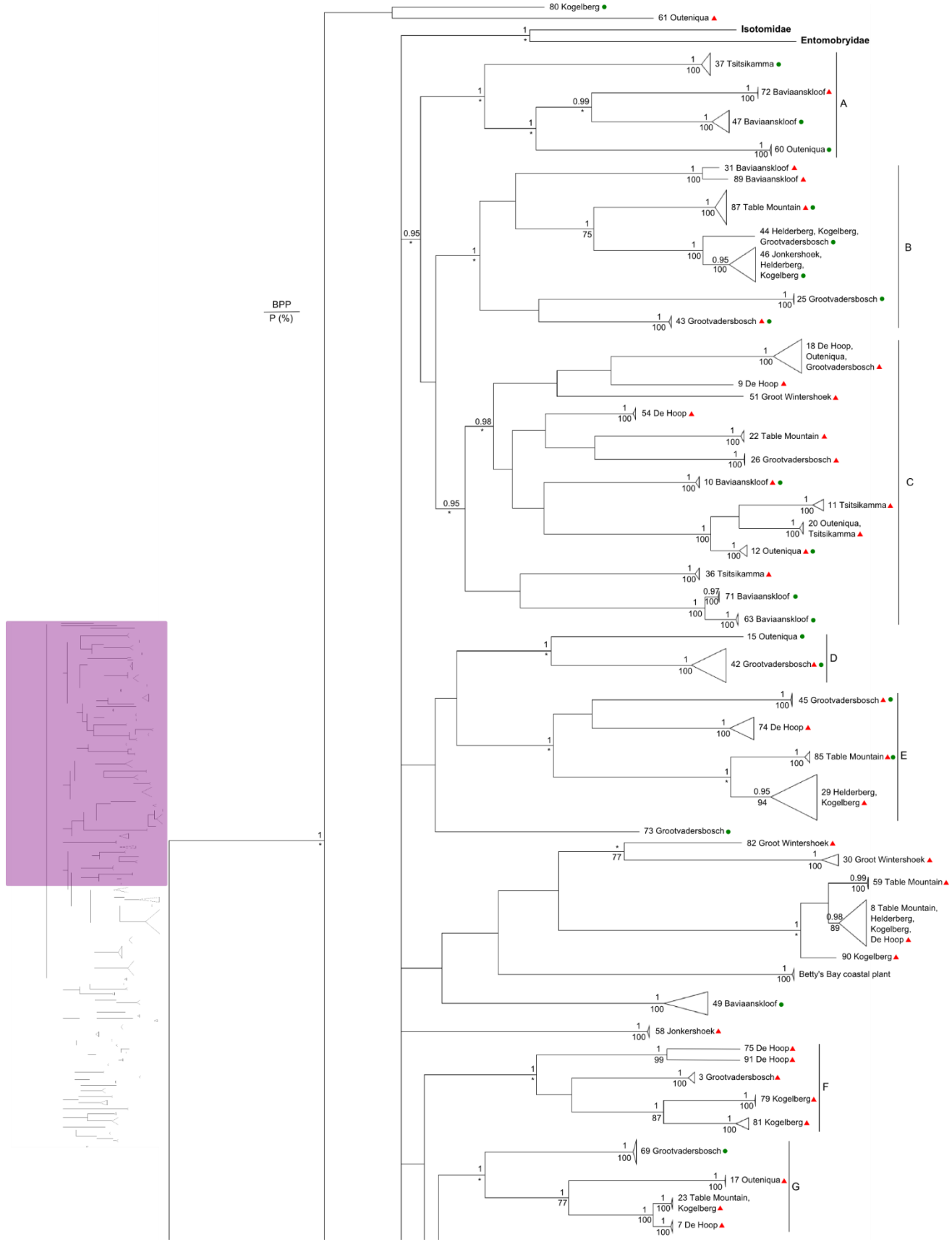



**Figure 2.3.** Histograms showing the frequency distributions of intra- and inter-MOTU sequence divergences of *Seira* with 469 individuals analysed. A) The 16 MOTUs with higher intra-specific divergences (>2.5%) were included; B) these MOTUs were excluded.



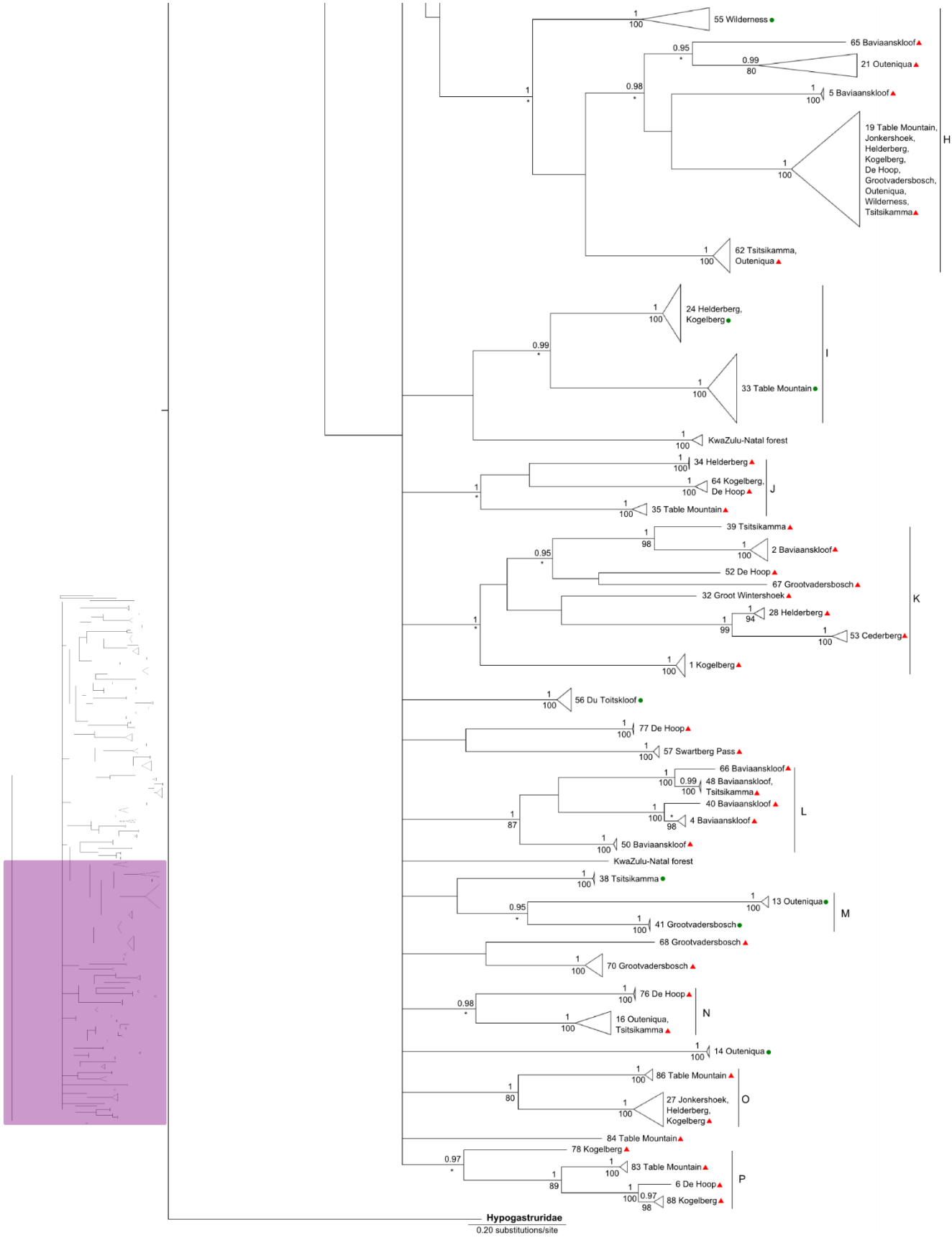



**Figure 2.4.** Bayesian phylogenetic tree derived from the COI nucleotide dataset illustrating the

phylogenetic relationships among *Seira* specimens from the Fynbos (▲) and Southern Afrotemperate forest (●). Entomobryidae, Isotomidae and Hypogastruridae were included as outgroup taxa. The 16 well-supported clades are highlighted with letters, and correspond with Figures 2.5 and 2.6. The nodal supports are indicated by the BPP and the P bootstrap values above and below the nodes, respectively. The BPP and bootstraps values of less than 0.95 and 75% are either indicated by asterisks or not shown in the tree. The terminal nodes were combined for each identified MOTU. MOTU codes correspond to Table 2.1. The section of the phylogeny shown on each page is highlighted in purple block on the left.

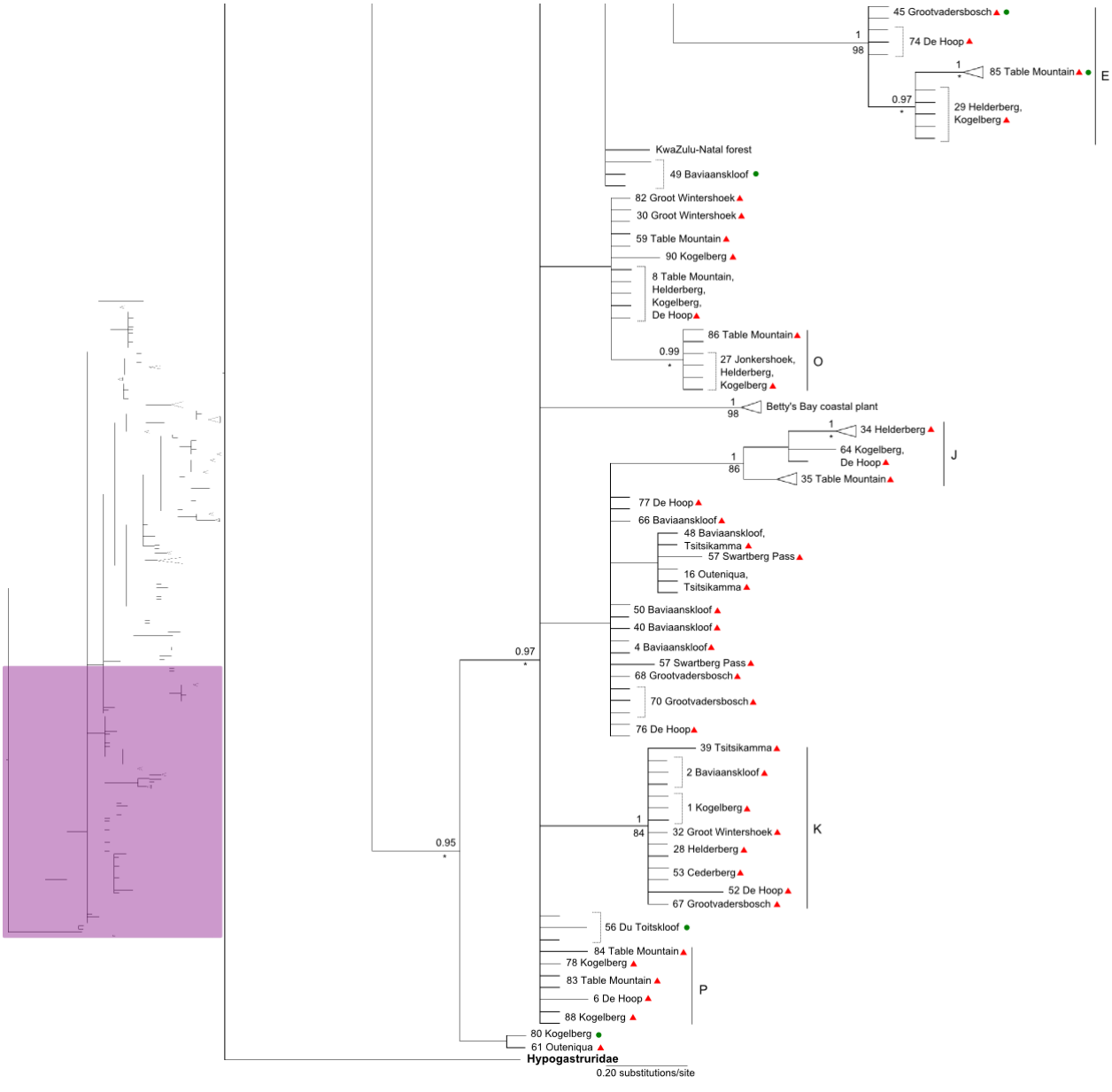
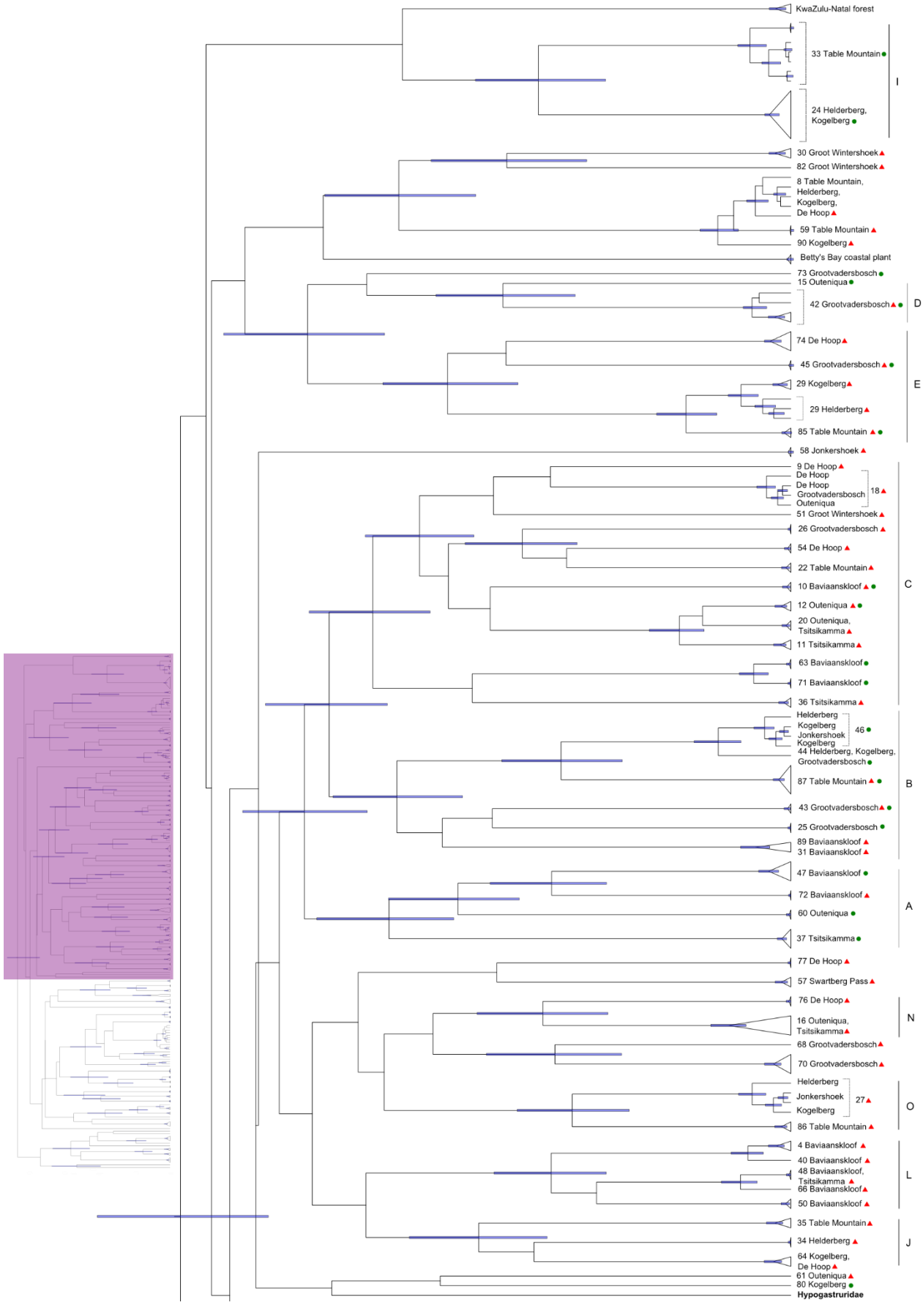



**Figure 2.5.** Bayesian phylogenetic tree obtained based on the translated amino acid dataset of genus *Seira* from the Fynbos (▲) and Southern Afrotemperate forest (●). The well-supported clades are highlighted with letters, and correspond with Figures 2.4 and 2.6. The statistical support of BPP and P bootstraps are indicated above and below the nodes, respectively. The BPP and bootstraps values of less than 0.95 and 75% are either indicated by asterisks or not shown in the tree. The terminal nodes were combined, where possible, for each identified MOTU. MOTU codes correspond to Table 2.1. The section of the phylogeny shown on each page is highlighted in purple block on the left.



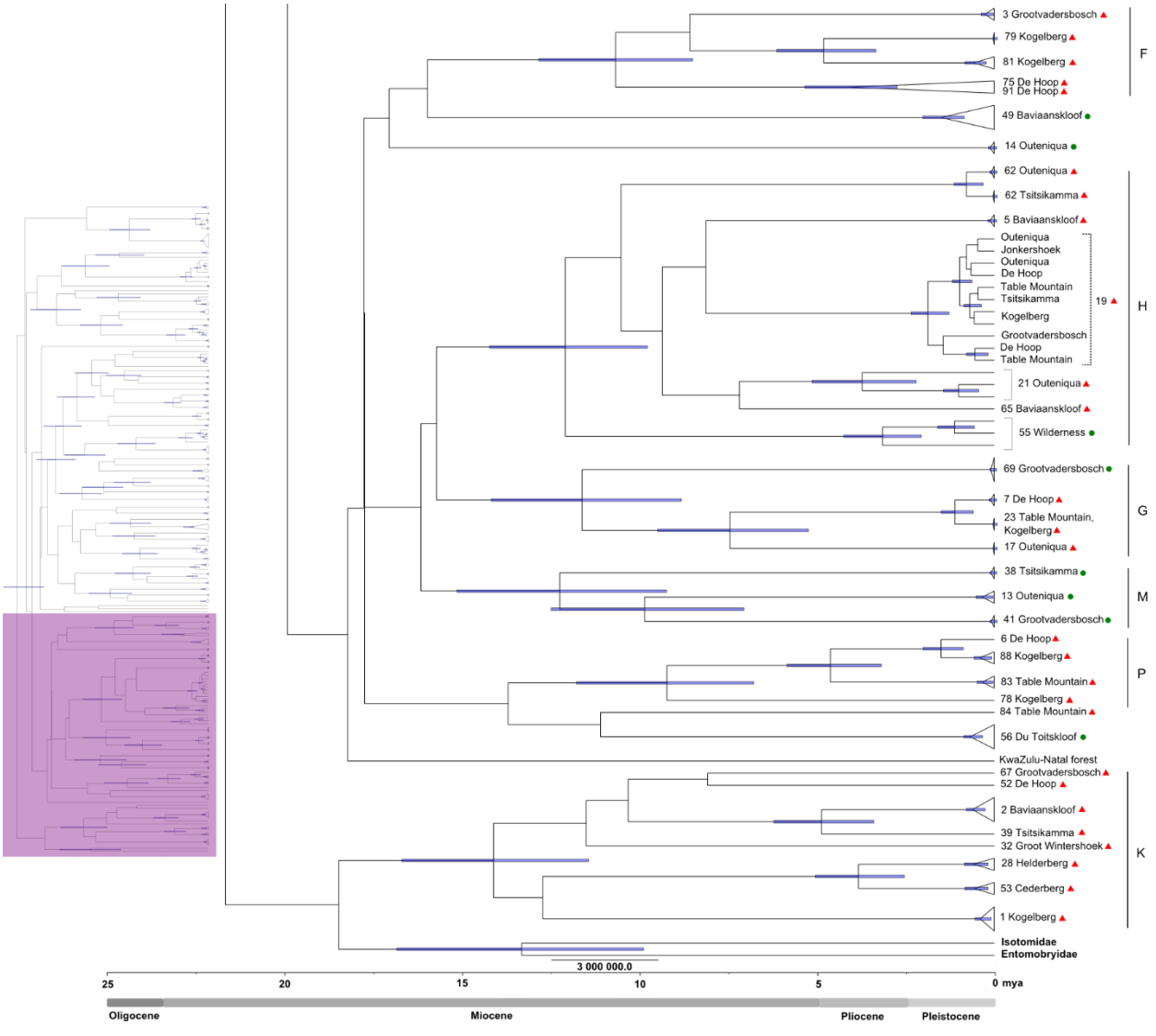



**Figure 2.6.** The dated phylogenetic reconstruction of genus *Seira* from the Fynbos (▲) and Southern Afrotemperate forest (●) habitat inferred from the COI nucleotide dataset. The best nodal age estimates were used to determine node depth. The blue bar at each node indicates 0.95 HPD intervals with divergence time in million years. MOTU codes correspond to Table 2.1. The section of the phylogeny shown on each page is highlighted in purple block on the left.

## Chapter 3

## Comparative diversity of the genus *Seira* (Collembola: Entomobryidae: Seirinae) in the Fynbos and Southern Afrotemperate forest remnants of the Cape Floristic Region


### Abstract

It is widely acknowledged that the richness of regional plant species is unusually high in the Cape Floristic Region (CFR), and this has been attributed to the remarkable turnover of species composition along the environmental and geographic gradients. It has been argued that the invertebrate diversity in this region is under-represented or species-poor compared to the exceptional plant diversity. However, recent studies on a broad range of insect groups have revealed that this may not be the case. Despite their importance in soil function, the spatial variation in soil faunal diversity has remained relatively poorly known. The aims of this chapter are to determine and compare the diversity patterns of one of the most diverse genera in the Collembola group, the genus *Seira* (Collembola: Entomobryidae: Seirinae) in the Fynbos and Southern Afrotemperate forest. Paired litter samples of both habitat types (Fynbos and forest) were collected in seven study sites, and unpaired samples in two additional Fynbos sites were collected. A combination of morphological- and molecular-based species discovery methods are used. Furthermore, eight abiotic variables (including soil chemical contents, temperature and relative humidity above the soil) were recorded and compared within, and between, habitat types. The relative effects of habitat types and study sites on the assemblage structure of *Seira* are analysed. The composition dissimilarity (beta diversity; $\beta_{sor}$) of assemblages in the Fynbos and forest habitats are calculated based on turnover- ($\beta_{sim}$) and nestedness-driven dissimilarity ($\beta_{sne}$). Analyses are done to determine the relationships between Simpson dissimilarity ($\beta_{sim}$) and geographic distance. The results revealed 5682 individuals putatively representing 89 *Seira* species (including nine cryptic species) from the Fynbos (67 species) and forest (32 species) habitats. The overall richness is four times as diverse as suggested by present taxonomic records, highlighting the current underestimation of species richness in the region. Fynbos has a significantly higher mean species richness than the forest for all sites combined and within the majority of individual sites. This could be due to the high availability of niches and resources created by the steep environmental and floristic gradients in the Fynbos habitats. However, species abundance is higher in the cooler and more humid forest habitats with soils that are richer in nutrients. The *Seira* assemblage composition differs significantly among habitat types and study sites, with variance explained by both variables. This suggests high levels of habitat specificity and localised distribution ranges of many *Seira* species. A high level of beta diversity with a low degree of nestedness is observed for both habitat types across all study sites.

Furthermore, *Seira* composition dissimilarity increases with increased geographic distance, with the south-western, southern and eastern sites clustering separately. The recorded environmental variables are significantly different between habitat types, contributing to the substantial differentiation of *Seira* assemblage composition among habitat types. Overall, habitat specialisation and restricted distribution ranges appear to have contributed to the high beta diversity in Fynbos and forest assemblages, as well as the high regional richness of *Seira* in the CFR.

## Introduction

Biodiversity is distributed heterogeneously across the globe, with some areas being substantially more species rich than others (Gaston, 2000). Three components are traditionally used to determine the community diversity at different spatial scales; namely alpha, beta and gamma diversity (Whittaker, 1960, 1972). Most research on biodiversity has focused primarily on the within-habitat diversity pattern (alpha diversity) and on regional (or gamma) diversity patterns. By contrast, empirical insights into the variability in species composition among sites in a given area (beta diversity) have been remarkably sparse until recently (Leibold et al., 2004), despite the obvious link that beta provides between local and regional diversity (Whittaker, 1960, 1972; Anderson et al., 2011).

One exception to this trend has been investigations of the Cape Floristic Region (CFR) of South Africa. The CFR is a globally recognised, major biodiversity hotspot, with extraordinarily high floristic endemism and species richness (Myers, 1990; Goldblatt, 1997; Goldblatt & Manning, 2002; Cowling & Pierce, 2004). In the CFR, substantial research has been undertaken into the patterns and mechanisms underlying the variations in plant diversity (e.g. Goldblatt, 1978; Cowling et al., 1992, 1996; Goldblatt, 1997; Goldblatt & Manning, 2002; Linder, 2003, 2005). It has been noted that the species richness of the flora is substantially higher than expected in the context of both its size and latitude (Cowling et al., 1996; Linder, 2003). The general conclusion is that the high regional plant species richness is mainly a consequence of substantial turnover in species composition along environmental and geographic gradients, while the overall local richness is not remarkable (Cowling et al., 1992, 1996; Simmons & Cowling, 1996; Goldblatt, 1997; Goldblatt & Manning, 2002). Several features of the CFR account for the high regional diversity, including the effects of relatively long-term climatic and geomorphic stability (Cowling et al., 2009), low gene flow, fire-associated niches, as well as a mosaic of complex and distinct climatic, edaphic and topographic conditions across the landscape (Goldblatt, 1997; Goldblatt & Manning, 2002; Linder, 2003).

Much of the plant diversity and endemism in the CFR is contributed by the most widespread and characteristic vegetation community, the fire-dependent Fynbos (Goldblatt, 1997; Goldblatt & Manning, 2002; Cowling & Pierce, 2004); while the more species-poor Southern Afrotemperate forest exhibits a highly fragmented and restricted distribution in this fire-prone ecosystem (Manders et al., 1992, Mucina & Geldenhuys, 2006). In contrast to the Fynbos, the forest species

richness increases toward the east (Lawrence, 1953), due to the more fertile soil and mesic conditions (Cowling & Holmes, 1992; Cowling et al., 1992). However, it has been proposed that most of the Fynbos environment is climatically suitable for the forest (Manders et al., 1992; Cowling et al., 1997), and that the dynamics of vegetation boundaries are mainly determined by the fire regime in relation to berg winds (Geldenhuys, 1994). Furthermore, the soils of forest habitats have a higher nutrient content compared to the Fynbos, and some of the differences in soil properties may be due to plant-induced effects together with the different nutrient cycling processes within the different communities (Manders et al., 1992). Therefore, the Fynbos and Southern Afrotemperate forest habitats differ substantially in terms of floristic composition (Campbell & Moll, 1977; Bergh et al., 2014), as well as community processes (Manders & Richardson, 1992); likely affecting the invertebrate communities in these vegetation types.

Despite the global significance of the CFR, current knowledge of the invertebrate diversity in this region is poor compared to other biomes in South Africa (e.g. Foord et al., 2008; Munyai & Foord, 2011; Haddad et al., 2013; Bishop et al., 2014). Early study suggested that the insect fauna richness is not particularly high in the CFR in comparison with other ecosystems in southern Africa, although the results varied among invertebrate taxa (Giliomee, 2003). However, work on particular plant specialist groups (such as the gall-forming insects and monkey beetles) (Wright & Samways, 1998; Colville, 2009) and some other groups has shown that this is not the case (Picker & Samways, 1996; Procheş & Cowling, 2006; Pryke & Samways, 2008, 2009a; Procheş et al., 2009; Braschler et al., 2012). Braschler et al. (2012) found that the local ant species richness in the Fynbos biome was within the range of global pattern, as predicted by the energy availability and climate. Similarly, at both local and biome scales, a comparable or higher level of species richness was found for a broad range of insect taxa between the Fynbos and selected adjacent South African biomes with similar plant species richness (Procheş & Cowling, 2006). On the Cape Peninsula, the surface-active invertebrates showed higher species richness and higher turnover in the Fynbos than the indigenous Southern Afrotemperate forests, presumably due to the heterogeneous abiotic characteristics of Fynbos habitats (Pryke & Samways, 2010). Furthermore, the composition of invertebrate assemblages was found to be significantly different between these two vegetation types (Koen & Breytenbach, 1988; Pryke & Samways, 2008, 2009b). Although the indigenous forests have emerged as less species rich in invertebrates, they do nonetheless appear to be important areas for many localised endemic invertebrates (Picker & Samways, 1996; Ratsirarson et al., 2002; Pryke & Samways, 2009a, 2010).

Despite the growing number of invertebrate diversity studies in the CFR, the soil fauna has largely been neglected; though its importance, at least in the forest habitats, was recognised early on (Lawrence, 1953). Consequently, the extent of soil faunal diversity, its spatial patterns, and the potential underlying mechanisms remain relatively poorly known. Some information is available on the ants (Botes et al., 2006; Braschler et al., 2012) and the dung beetles (Sole et al., 2005; Sole & Scholtz, 2013). However, for groups of smaller body size, which play significant roles in ecosystem processes and services, much less is known. To rectify this situation, a large study of the Collembola faunas of the region was initiated as part of the Barcode of Life Project (Hebert et al., 2003; Ratnasingham & Hebert, 2007). Collembola is a hexapod group of substantial ecological importance in both above- and below-ground ecosystems (Wardle et al., 2004; De Deyn & Van der Putten, 2005; Bardgett & Van der Putten, 2014). They are one of the most abundant and diverse invertebrates in most terrestrial ecosystems globally (Hopkin, 1997; Rusek, 1998; Deharveng, 2004), including the CFR (Janion et al., 2011b). The CFR component of the overall Collembola research is concerned with several key areas. These include the assessment of soil system functioning (Bengtsson et al., 2011, 2012), their diversity (Janion et al., 2011b; Janion-Scheepers et al., 2015), and the extent to which the fauna includes invasive alien species (Janion et al., 2011b), how these are distributed (Liu et al., 2012), and the effects of these on system structure and functioning (Leinaas et al., 2015). Because of the considerable diversity of the fauna, and suggestions of dramatic radiations in genera such as *Parisotoma* (Potapov et al., 2011), *Ectonura* (Janion et al., 2011a) and *Seira* (Janion et al., 2011b), a major focus of the work has been on understanding the diversity in these genera and their spatial patterns across the CFR; including the extent of beta diversity and habitat specificity. Collembola are not thought to have intimate associations with plants, but are known to be sensitive to micro-environmental changes (Choi et al., 2002; Krab et al., 2010; Chagnon et al. 2001; Wu et al., 2014). They are therefore ideal organisms for providing assessments of the extent to which drivers affecting plants may also contribute to diversity variation in other groups in the CFR (Janion, 2013).

Much work has examined Collembola spatial diversity variation and its correlates in other parts of the world, although mostly in the Northern Hemisphere (e.g. Deharveng, 1996; Vanbergen et al., 2007; Raschmanová et al., 2008, 2013; Robson et al., 2009; Sabais et al., 2011; Zeppelini et al., 2013; Heiniger et al., 2014). Species richness and abundance of Collembola are affected by edaphic factors, such as soil moisture content (Robson et al., 2009; Zeppelini et al., 2013), temperature, pH (Vanbergen et al., 2007; Raschmanová et al., 2013), organic matter content (Hasegawa, 2001; Heiniger et al., 2014) and exchangeable cations (Raschmanová et al., 2008;

Treasure & Chown, 2013). Moreover, species compositional turnover increases with increasing habitat heterogeneity at the landscape scale (Vangergen et al., 2007). Assemblage composition also differs significantly across different vegetation types (Robson et al., 2009; Bolger et al., 2013; Zeppelini et al., 2013). However, investigations of spatial diversity have been uncommon in South Africa until recently (Janion et al., 2011b; Liu et al., 2012). For the CFR only a single study of factors influencing spatial diversity variation of Collembola has been undertaken at the species level (Liu et al., 2012). It was found that morphospecies richness and abundance were significantly and positively correlated with relative humidity and temperature, and the assemblage composition was substantially different among habitat types.

The aim of the present study, therefore, is to investigate spatial variation in diversity of one of the most diverse genera of Collembola in the CFR: *Seira* (Lubbock, 1870). By sampling two different vegetation types (Fynbos and Southern Afrotemperate forest) across the south-western and south-eastern regions of the CFR, variation in the major spatial components of diversity of the genus, and their habitat specificity can be assessed, so providing a comparison with other plant and invertebrate taxa studied in the region.

As part of the largest Collembola family, Entomobryidae (Bellinger et al., 2015), the genus *Seira* is almost ubiquitous in terrestrial habitats worldwide (Christiansen & Bellinger, 2000), with a clear radiation in the CFR (Yosii, 1959; Janion et al., 2011b). Only 29 species have been described from South Africa (Börner, 1908; Womersley, 1934; Paclt, 1959, 1967), of which 21 species are reported for the Western Cape (Yosii, 1959; Coates, 1968). However, recent sampling has revealed that this genus may be more diverse than previously expected (Janion et al., 2011b). Therefore, I used a combination of morphological- and molecular-based species discovery methods (Hogg & Hebert, 2004) to provide a more robust assessment of diversity in the understudied genus *Seira*.

The objectives were to determine: (1) the diversity of *Seira* from two contrasting habitat types (Fynbos or Southern Afrotemperate forest) within each study site (alpha diversity); (2) the spatial turnover and nestedness components of *Seira* assemblages between Fynbos and Southern Afrotemperate forest habitat within/among sites, as well as among the same habitat type between sites (beta diversity); (3) the total diversity of *Seira* in the CFR, across both of the habitat types

(gamma diversity); and (4) the differences in the environmental attributes associated with each habitat type.

*Hypotheses*

1. The species richness and beta diversity of *Seira* species is predicted to be higher in the Fynbos habitats than that of the relatively plant species-poor forests, because the pronounced environmental heterogeneity of Fynbos habitat may favour high faunal diversity and turnover (Pryke & Samways, 2009a) by providing diverse habitats. However, *Seira* abundance is expected to be higher in the more homogenous forest habitats, as previous studies have shown positive relationships between soil moisture or chemical contents and Collembola abundance (Raschmanová et al., 2008, 2013; Robson et al., 2009; Zeppelini et al., 2013).

2. Owing to the differences in plant community composition and environmental conditions between the Fynbos and Southern Afrotemperate forest, the beta diversity of *Seira* is expected to be mainly attributed to the turnover of species among these habitat types. Furthermore, the dissimilarity of *Seira* assemblages should be positively correlated with geographical distance as they are considered to geographically localise. Therefore, high beta diversity is likely to be characterised by a high incidence of habitat specialised or localised *Seira* species.

3. The environmental conditions (temperature, relative humidity and soil parameters) are expected to be significantly different between Fynbos and Southern Afrotemperate forest habitat.

## Materials and methods

*Study sites*

This study was undertaken in the indigenous Fynbos and Southern Afrotemperate forest habitats of the Cape Floristic Region (CFR) of South Africa. This region is characterised climatically by a wide variation of rainfall seasonality. A winter rainfall regime characterises the extreme southwest section of the region, while the eastern section lies in a summer rainfall-dominated area (Goldblatt, 1978; Linder, 2003; Rebelo et al., 2006). The precipitation pattern in the Southern Cape is aseasonal, with spring and autumn maxima (Linder, 2003; Rebelo et al., 2006). The mean annual precipitation for Fynbos varies between 250 mm and 650 mm, with the highest record of 1 000 mm per year in the mountains of southwest and southern Cape (Manning, 2007). The average mid-summer temperature ranges between 15 and 25°C. However, inland valleys can reach high 30°C or low 40°C values (Manning, 2007). The most prevalent vegetation is the sclerophyllous, fire-adapted shrubland called Fynbos (Figure 3.1), which covers 56% of the area of CFR. It comprises of about 83% of the plant species, of which over 80% are endemic to the Region (Rebelo et al., 2006). Fynbos generally thrives on acidic infertile sandy soils, and less often on soils derived from limestone, shale- and granite, or duricrust outcrops and alluvial sediments (Rebelo et al., 2006). Fynbos is structurally represented by the occurrence of three dominant plant types including restiods, ericoids, and the overstorey proteiod shrubs (Rebelo et al., 2006).

The Southern Afrotemperate forest unit encompasses the Western Cape Talus forest, the Western Cape Afrotemperate forest and the Southern Cape Afrotemperate forest (Mucina & Geldenhuys, 2006). The distribution of forest remnants is generally widely separated and localised across the Western and Eastern Cape, embedded within other large-scale biomes, such as Fynbos (Mucina & Geldenhuys, 2006). The forest patches are typically less than 100 hectares (ha) in size (Mucina & Geldernhuys, 2006). The most extensive forest complex (25 706 ha) is found on the sheltered plateaux, coastal scarps and seaward slopes in the Knysna-Tsitsikamma region of southern Cape (Mucina & Geldernhuys, 2006). The western forest is primarily confined along the bottom of south- and east-facing slopes and in the fire refuge sites (i.e. ravines, deep gorges or along stream banks) of the Cape Fold Belt mountains (Mucina & Geldernhuys, 2006). The substrates supporting the forests are mainly derived from the sandstone of Table Mountain Group and Cape Supergroup shales, as well as the Cape Granite (Mucina & Geldenhuys, 2006). The derived soil form ranges from sandy humic to shallow and skeletal (Mucina & Geldenhuys, 2006). The mean annual precipitation is approximately 863 mm and the mean annual temperature

is approximately 16.7 ˚C in Southern Afrotemperate forest (Mucina & Geldenhuys, 2006). This forest group is mainly comprised of yellowwoods, stinkwood, ironwood, cherrywood, white alder and others, while different plant species dominate the scree and deep-ravine habitats (Mucina & Geldenhuys, 2006). The understory vegetation is well-developed, particularly in cool and humid habitats (Mucina & Geldenhuys, 2006) (Figure 3.1).

A total of nine study sites were selected to cover a broad geographical extent of both the Fynbos and Southern Afrotemperate forest. These sites were Groot Wintershoek, Table Mountain National Park, Helderberg, Kogelberg, De Hoop Nature Reserve, Grootvadersbosch Nature Reserve, Outeniqua Nature Reserve, Tsitsikamma National Park and Baviaanskloof (Figure 3.2). Seven of them had both habitat types, however forest was not represented in Groot Wintershoek and De Hoop Nature Reserve.

*Sampling*

Sampling took place between early October 2012 and early April 2013 during the warm season to exclude any seasonal-related changes in assemblage structure. In addition, the activity and diversity of *Seira* appears to be highest during this period (C. Janion per. comm.). Four replicated, mature patches (> 5 years) were considered for each habitat type per study site, and the distances between patches were at least 500 m. Sampling was undertaken away from the transitional area at each side of the vegetation patch to avoid the edge effects caused by neighbouring vegetation.

In each patch, a maximum of two transects were marked out with a distance of 250 m between them, and six sampling points were spaced at 20 m intervals along each transect (Figure 3.3). Global Positioning System (GPS) coordinates were recorded for the location of each sampled point as sampling information. A 500 ml plastic container of plant litter/soil was collected by a spade at the shaded side of each sampled point and sealed in a plastic container, thus providing 12 litter samples per patch. This constituted a total of 48 litter samples per habitat type in a site. Due to the highly fragmented nature of forest patches and the limited access to these patches, the number of transects per patch and the distance between sampled points were different for two sites: Helderberg and Kogelberg had only 42 and 36 litter samples of the Southern Afrotemperate forest, respectively. One of the Helderberg forest patches had one transect of six sampled points, while two of the Kogelberg forest patches each consisted of one transect of six

sampled points. The sampled points along these transects were placed 10 m apart. The sampling events covered a large extent of Southern Afrotemperate forest in the region, therefore the collected samples can thus be regarded as representative of the habitat even though the overall forest sample size was smaller than the Fynbos.

The collected litter samples were stored under cool conditions for no longer than four days before extraction in the laboratory. Each sample was extracted individually into 99.9% ethanol using the Berlese-Tullgren funnel extraction method (Hopkin, 2007, Liu et al., 2012), and this was carried out as soon as possible after collection to minimize natural death and the risk of Collembola being consumed by arthropod predators such as spiders, mites and beetles (Rusek, 1998; Hopkin, 2007). The extraction was conducted over one week or until the litter was dried at room temperature. This extraction method has 90% effectiveness as the litter/soil invertebrates tend to migrate to the bottom layer of organic material and fall into the plastic vial to avoid desiccation and heat (Hopkin, 2007).

*Environmental attributes*

Seven abiotic variables were used to assess the environmental conditions associated with each habitat type (Fynbos and Southern Afrotemperate forest). The ambient temperature (°C) and relative humidity (%) were taken directly above the litter/soil ground on the shaded side of each sampled point by using a handheld Vaisala HM32 thermohygrometer (Vaisala, Vantaa, Finland). In addition, one soil sample of 1 kg was collected haphazardly at the sampled points (soil depth: 10 cm) along each transect per site, resulting in a sample size of eight for each habitat type for most of the study sites, with the exception of Helderberg and Kogelberg forest habitat. The soils were analysed for pH (McLean, 1982), Phosphorus (P) (Bray & Kurtz, 1945), Carbon (C) (Nelson & Sommers, 1982), Nitrogen (N) and Magnesium (Mg) (Chapman, 1965) by BemLab (Pty Ltd.), Somerset West, South Africa.

*Species identification*

Specimens from the genus *Seira* were sorted from the extracted samples and assigned into morphospecies on the basis of their external characteristic features (e.g. colour/pigment pattern) or identified to morphospecies level based on the taxonomic keys available (Börner, 1908; Womersley, 1934; Coates, 1968; Paclt, 1959, 1967; Yosii, 1959; Bellinger et al., 2015) and information collected by a collaborative project in the Centre of Excellence for Invasion Biology

(SA-France bilateral grant 2008-2012 in collaboration with the Museum National d'Histoire Naturelle (MNHN) Paris) (Janion et al., 2011b). The sorting of specimens was done by one researcher (WPAL) using a Leica MZ7.5 dissection microscope to ensure the recognition consistency in the morphospecies identification process. In addition, the delimitation of morphospecies was assessed by Dr. C. Janion-Scheepers, who has considerable experience with the South African Collembola fauna (Janion et al., 2011b). For each sample, the species richness (the total number of species present), species abundance (the number of individuals per species) and species identity were recorded, as these are the most prominent forms of diversity. The samples are deposited in the Centre of Invasion Biology, University of Stellenbosch, South Africa.

*Barcoding*

It has been found that DNA barcoding provides a useful complement to conventional morphological taxonomy (Hogg & Hebert, 2004), because it straddles the middle ground between available taxonomic expertise and the demand for identification capability (Tautz et al., 2003). This enables rapid assessment of biodiversity (Hajibabaei et al., 2007), which is particularly useful in hyper-diverse and poorly explored taxonomic groups, such as the Collembola. Here, four specimens per morphospecies were used for DNA barcoding, and these specimens were individually photographed for morphological voucher using a Leica MZ7.5 stereo microscope and a Leica DFC320 digital camera. They were then transferred to uniquely barcoded 96-well glass fibre plates (95 specimens, one control) with 99% ethanol. The plates were processed by the Biodiversity Institute of Ontario, Guelph, Canada for DNA extraction and sequencing. The focal region used for the Barcode of Life Data System (BOLD, http://www.boldsystems.org/) is the 5' end of the mitochondrial cytochrome oxidase subunit I (COI) (Hebert et al., 2003; Ratnasingham & Hebert, 2007). The extraction and amplification of COI were performed using the standard protocol described by the Canadian Centre for DNA Barcoding (CCDB, http://www.ccdb.ca/). The procedure is described in more detail in Chapter two. Sequences, taxonomic and collection information, as well as the photographs of the specimens were deposited in the BOLD Identification System under the project entitled Collembola of South Africa (COLSA). After the DNA extraction, the recovered vouchers were stored in 99% ethanol for reference, as the chaetotaxy characteristics can be used in further morphological examination when necessary (Porco et al., 2010b). The reference collections will be deposited at the Centre of Invasion Biology (Stellenbosch University) and the Iziko Museum, Cape Town.

*Statistical approach*

*Tourists*

Tourist species are transient individuals in a given habitat type without intimate or lasting associations with that particular habitat (Moran & Southwood, 1982). It has been suggested that they can lead to overestimations in the total number of species within habitats (Gaston et al., 1993), thus biasing species occurrence estimates across habitats (Mercer et al., 2000). Tourist species in this study were identified and excluded from the data analyses. There is no general accepted benchmark in identifying tourist species using quantitative methods based on the abundance data (see Chown & Steenkamp, 1996; Mercer et al., 2000). In this study, the proportion of sum method described by Mercer et al. (2000) based on the abundance data was followed. A species was regarded as a tourist in a given habitat type when its abundance in that habitat was represented by less than 5% of its total abundance across both habitat types within a study site. However, it is important to distinguish between tourist and rare species in an assemblage of a given habitat (Mercer et al., 2000). Therefore, even though a species was identified as a tourist based on the quantitative method, it was rather considered as rare if it was recorded in more than 60% of the samples collected from a particular habitat type (see Dufrêne & Legendre, 1997).

*Cryptic species*

Cryptic species are defined as more than one distinct species that are inaccurately classified and hidden under a single nominal species name, as they are morphologically indistinguishable (Bickford et al., 2006). It is believed that the existence of cryptic species is one of the reasons for the current underestimation of true Collembola species richness (e.g. Deharveng, 2004; Hogg & Hebert, 2004; Porco et al., 2010a). Thus, accurate discrimination of Collembola species in cryptic complexes is crucial in the study of biological diversity. Two different approaches could be used in the data analyses of this chapter, namely: (1) the conservative approach with the exclusion of cryptic species, and (2) the liberal approach with the inclusion of cryptic species, and the number of individuals divided evenly between the cryptic species of that particular nominal species. Here, the distinct genetic entities (Molecular Operational Taxonomic Units – MOTUs; Floyd et al., 2002; Blaxter, 2004) were identified following a DNA barcoding approach (Hebert et al., 2003; Hogg & Hebert, 2004; Porco et al., 2012, 2014), tree-based criteria and nucleotide pairwise divergence thresholds, which are described in detail in chapter two. In this chapter, I used the term species as replacements for MOTUs, to refer to these identified genetic entities based on the molecular

data, because most of the diversity studies are given with reference to a species. However, it is emphasised that the membership of these MOTUs is a working hypothesis that requires further detailed taxonomic work and molecular testing, prior their formal identification as individual biological species.

*Local diversity*

Sample-based rarefaction curves were compiled separately for the habitat types (Fynbos and Southern Afrotemperate forest) for each study site to evaluate the degree to which sampling was complete, using EstimateS V 9.1.0 (Colwell, 2013, http://viceroy.eeb.uconn.edu/estimates; Colwell & Elsensohn, 2014). The effectiveness of sampling is deemed as adequate when the sample-based rarefaction curves and richness estimators approach a plateau beyond a particular number of samples (Longino et al., 2002), as no further species in an assemblage will be added (Gotelli & Colwell, 2001). Here, the sample order of each plot was randomised 500 times without replacement as all the samples in the dataset were included to compute the estimators of asymptotic species richness. The Chao1 (Chao, 1984) and the second-order Jackknife (Burnham & Overton, 1978) richness estimators for abundance data were used to evaluate sampling adequacy, as the Chao and Jackknife estimators usually seem to perform better than the other estimators in estimating the number of undetected species from the number of rare species in an assemblage (Walter & Martin, 2001; Walter & Moore, 2005). The Chao estimators have found to be most precise and least biased, followed by the Jackknife estimators (Walter & Martin, 2001).

*Among-site diversity*

To examine the Fynbos and Southern Afrotemperate forest assemblage structure of the genus *Seira*, a non-parametric multivariate approach, as described by Clarke (1993), was implemented in PRIMER V 6.0 (Plymouth Routine in Multivariate Ecological Research, see Clark & Gorley, 2006). Species richness and abundance were pooled across the samples per patch within the seven sampled sites with both habitat types prior to the analyses. The abundance data were double square-root transformed to weight the common and rare species equally (Clark & Gorley, 2006). The Bray-Curtis similarity coefficient measure was used to construct a similarity matrix among sampled patches and study sites. A two-way nested Analysis of Similarity (ANOSIM), with habitat types nested within sites, was used to determine the effects of habitat types and study sites on the assemblage structure of *Seira*, and to identify which of these factors had the most influence on the assemblages in the CFR. The ANOSIM procedure returns a Global *R* statistic

that is a measure of difference among groups (0 and 1 represent complete mixing and distinct separation, respectively), and a *P* value to determine the significant differences of the analyses (Clark & Warwick, 2001). The differences in *Seira* assemblage composition among study sites and habitat patches were graphically depicted in ordinations by non-metric multi-dimensional scaling (nMDS) using 500 random restarts based on the Bray-Curtis similarity index. A stress value (measure of distortion) of less than 0.1 indicates the ordination is a good representation of the level of similarity among samples (Clark & Warwick, 2001). The analyses were repeated after transformation of the species abundance data to presence-absence to determine if the significant differences in assemblages between habitat types and study sites could be explained by the variations in species richness and identity rather than by the difference in abundance between species (see Liu et al., 2012). The differences in *Seira* assemblage structure among study sites and patches were re-analysed after the exclusion of extreme outliers on the nMDS plots.

To investigate the compositional dissimilarity of *Seira* assemblage between Fynbos and Southern Afrotemperate forest habitat within and among study sites, the beta diversity (Sørensen's index of dissimilarity, $\beta_{sor}$) was separated into two distinct components: turnover- (Simpson dissimilarity, $\beta_{sim}$; Lennon et al., 2001) and nestedness-driven dissimilarity ($\beta_{sne}$) (Baselga, 2010, 2012):

$$\beta_{\mathrm{sor}} = \beta_{\mathrm{sim}} + \beta_{\mathrm{sne}} \equiv \frac{b+c}{2a+b+c} = \frac{b}{b+a} + \left(\frac{c-d}{2a+b+c}\right)\left(\frac{a}{b+a}\right)$$

a = the number of shared species between two cells

b = the number of species unique to the poorest site

c = the number of species unique to the richest site

Briefly, turnover implies that the species in one site are replaced by different species in the other site, while nestedness occurs when sites with the species-poor assemblage are subsets of sites with species-rich assemblages (Baselga, 2010, 2012). Species abundance data was transformed as presence-absence per habitat type in the individual study site (excluding sites with single habitat type – De Hoop and Groot Wintershoek) prior to the analyses. The measure of composition dissimilarity ranges from 0 to 1, where 0 represents compositionally identical assemblages and 1 indicates no species shared between the assemblages. First, I calculated the multiple-site

dissimilarity for the assemblages in Fynbos, forest independently, and as well as both habitat types combined. Their patterns of spatial turnover ($\beta_{SIM}$), nestedness ($\beta_{SNE}$) and total beta diversity ($\beta_{SOR}$) were compared.

Second, pairwise dissimilarity measures were calculated to determine the increase of *Seira* compositional dissimilarity with geographic distance. Dendrograms of the turnover ($\beta_{sim}$) and nestedness ($\beta_{sne}$) were plotted using the average linkage to allow for the identification of dissimilarity patterns between habitat types and study sites. Mantel permutation tests (999 permutations) were performed using the Spearman's rank correlation to determine the existence of significant relationships between Simpson dissimilarity ($\beta_{sim}$) and geographic distance for Fynbos, forest and both habitat types combined of all study sites. Euclidian distances for the distance matrix were calculated from the recorded longitudinal and latitudinal coordinates of each habitat type within individual study site. Following Baselga (2012), capital letters for subscripts were used to differentiate the multiple-site dissimilarity measures from the pairwise dissimilarity. The beta diversity and graphs were implemented in R statistical software 3.2.1 (R Development Core Team, 2015) by means of the 'betapart' package 1.3 (Baselga & Orme, 2012), and the Mantel test was implemented using the 'vegan' package 2.3-0 (Oksanen et al., 2015).

To detect the overall differences in the species richness and abundance of *Seira* among the Fynbos and forest habitats of all study sites combined (excluded De Hoop and Groot Wintershoek), Generalized Linear Mixed Models (GLMM) assuming a Poisson error distribution and logarithmic link function were applied. This is suggested for count data when the response variables and model residuals are not normally distributed. However, the mixed model with the abundance data was fitted with Negative Binomial error distribution to account for the overdispersion as indicated by the large ratio of the residual deviance to the residual degrees of freedom. Habitat types (Fynbos and forest) were treated as the independent variable (fixed effect) with the study sites as the random effect. The GLMMs were implemented by means of the *glmer* function in the 'lme4' R-package 1.1-7 (Bates et al., 2014). Furthermore, Generalised Linear Models (GLM) were used to separately test for the effect of habitat types on the species richness and abundance of *Seira*. Habitat types were the only categorical predictor variable, without consideration for the random effect because there was no study site effect within each site. Poisson error distributions with logarithmic link were used in the analyses of richness, while the quasi-Poisson approach was used in the model for the overdispersed abundance data in R 3.2.1

(R Development Core Team, 2015). The GLMMs and GLMs were carried out based on the total species richness and total abundance in each patch across the transect/s of study sites.

*Environmental attributes*

Correlations between temperature and relative humidity were analysed using Pearson's product-moment correlation coefficient (*r*). GLMMs were constructed incorporating the habitat types as the fixed effect and the study sites as the random effects, to compare the overall temperature and relative humidity between Fynbos and forest habitat. The Gamma distribution errors with identity link were used in the models, as it is proposed for continuous and positive response variables with right-skewed distributions (Crawley, 2007). The same GLMM approach (as above) was applied for the comparisons of soil parameters (pH, Mg, P, C and N) between the habitat types. For individual study sites, GLMs (Gamma errors and identity link) with habitat types as factor were performed to determine the patterns of abiotic factors between Fynbos and forest. All analyses were conducted in R 3.2.1 (R Development Core Team, 2015), and the GLMMs were implemented by means of the *glmer* function in the 'lme4' package 1.1-7 (Bates et al., 2014).

## Results

A total abundance of 5682 individuals representing 89 *Seira* species were collected from the Fynbos and Southern Afrotemperate forest habitats in the CFR. In most cases, the molecular analyses based on the COI barcoding gene supported the divisions based on morphology. However a small number of discrepancies suggest that what was previously considered as four species based on morphology were actually nine morphologically cryptic species. They occurred in the Fynbos habitats of Helderberg, De Hoop, Grootvadersbosch, Outeniqua and Tsitsikamma, with two cryptic species in each site and the latter two sharing one cryptic species. The 6% difference of total species richness between the inclusion and exclusion of cryptic species did not significantly affect the statistical results, therefore only the liberal approach (see Materials and Methods) was used in the data analyses. Based on the criteria for tourists, 39 individuals representing eight *Seira* species were regarded as tourist species. Six of these species (35 individuals) were tourists in the Fynbos habitat, whereas four individuals (two species) were tourists in the forest habitat. The tourist species represented less than 1% of the total abundance of *Seira* in collected samples across the study sites.

Of the 89 species collected, 57 species (2685 individuals sampled) were exclusively found in Fynbos (Table 3.1), and of these 50 species appeared to have a very narrow distribution (i.e. present in one study site), whereas seven species occurred in more than a single site. The forest taxa comprised 22 unique *Seira* species (2997 individuals sampled (Table 3.1)), of which 20 species had extremely localised distributions (confined to one site) and the distribution ranges of two species were broader than a single site. Ten species were shared between the two habitat types, but only within the same study site. The mean species richness across all the study sites was significantly higher (i.e. two fold) in the Fynbos than the forest habitats (mean ± SE, Fynbos = 4.857 ± 0.348, forest = 2.679 ± 0.236; $z$ value = 4.148; $P < 0.001$) (Figure 3.4A; Table 3.2), however there was no significant difference in the abundance of *Seira* between these two habitats (mean ± SE, Fynbos = 74 ± 13.730, forest = 107.036 ± 17.732; $t$ value = -1.505; $P < 0.001$) (Figure 3.4B; Table 3.3). Similar findings were obtained for the mean species richness within individual study sites (Figure 3.4A; Table 3.2). In addition, the majority of the sites had higher mean abundance in the forest habitat than the Fynbos, with the exception of Helderberg and Kogelberg (Figure 3.4B; Table 3.3).

The sample-based rarefaction curves and richness estimators reached an asymptote for some of the Fynbos and most of the forest habitats, indicating that a substantial portion of the *Seira* species had been captured (Supplementary Figure S3.1 and S3.2). In most study sites, the rarefaction curves and estimators reached asymptotes well before the highest observed species richness for the forest habitat (Supplementary Figure S3.2). Therefore, the sampling of these sites was considered adequate and the species richness estimates were regarded as reasonable representatives. However, this was not found in six of the Fynbos habitats, as there were differences between the expected species richness and second-order jackknife richness estimators; suggesting that the observed species richness in those Fynbos sites may have been underestimated (Supplementary Figure S3.1).

Seven extreme outliers were found: four were from the Tsitsikamma forest patches and three from the Baviaanskloof forests. A two-way nested ANOSIM based on the species abundance data with outliers revealed that the *Seira* assemblage composition differed significantly among habitat types (i.e. Fynbos and forest) (Global $R$ = 0.838, $P$ = 0.001) and among study sites (Global $R$ = 0.588, $P$ = 0.001) (Figure 3.5A). Similarly, significant differences were found among habitat types (Global $R$ = 0.842, $P$ = 0.001) and sites (Global $R$ = 0.599, $P$ = 0.001) based on the species identity of *Seira* assemblages (with outliers; Figure 3.5C). When the analysis was repeated without the outliers, the effect of habitat types (species abundance: Global $R$ = 0.889, $P$ = 0.001; species identity: Global $R$ = 0.882, $P$ = 0.001) and study sites (species abundance: Global $R$ = 0.692, $P$ = 0.001; species identity: Global $R$ = 0.701, $P$ = 0.001) on the composition of *Seira* assemblages remained significant (Figure 3.5B and D). The differences among remaining patches were clearly shown without some of the overlaps among them. Despite the fact that the majority of the variance in species assemblage structure (based on both species identity and abundance) appeared to be explained by the different habitat types, a significant amount of variance was also explained by study site. The stress value in each of the nMDS ordinations was low (≤ 0.010), suggesting that the plots were excellent representations of the level of similarity between each sampled patch of the *Seira* assemblages. Although *post-hoc* tests of hypotheses cannot be undertaken using the ANOSIM approach, the nMDS plots (Figure 3.5) revealed a prominent geographic association of sites. Those in the southwest (Table Mountain, Helderberg, Kogelberg and Grootvadersbosch) clustered together, as did those in the south and east (Outeniqua, Tsitsikamma and Baviaanskloof), with some possible overlap indicated by Grootvadersbosch.

In the multiple-site dissimilarity analyses using the Sørensen's index, the estimated beta diversity ($\beta_{SOR}$) of *Seira* species was mainly due to the spatial turnover ($\beta_{SIM}$), with a very low degree of nestedness ($\beta_{SNE}$), as might be expected based on single site occurrences and habitat specificity (Table 3.4). This pattern was consistently found within each habitat type (Fynbos and forest) across study sites, as well as for both habitat types combined across sites (Table 3.4). The beta diversity was similar for the Fynbos ($\beta_{SOR}$ = 0.962) and forest ($\beta_{SOR}$ = 0.971), suggesting over 96% species turnover between sites of the same habitat. In some instances, forest and Fynbos habitats from the same site had more similar *Seira* assemblages; whereas in other instances similarities in *Seira* composition appeared to be linked by habitat type (Figure 3.6A), suggesting that both of these factors play an important role in determining assemblage composition. The pairwise analyses yielded the patterns of compositional dissimilarity between habitat types and study sites derived from species replacement ($\beta_{sim}$) and nestedness ($\beta_{sne}$) components of dissimilarity (Figure 3.6A and B). Figure 3.6B suggested that very few sites share species and consequently no patterns of nestedness could be discerned. Supporting the above mentioned nMDS plots, for both habitat types combined, the Mantel analyses showed that there was a significant positive correlation between the pairwise measure of $\beta_{sim}$ with geographic distance ($r$ = 0.489; $P$ = 0.001) for the *Seira* assemblages: this suggests that the closer sites are together, the more similar their *Seira* assemblages. Similarly, in separate analyses for the habitat types, a strong positively correlated pattern between proximity and assemblage similarity was found in the Fynbos habitats across study sites ($r$ = 0.762; $P$ = 0.001). This pattern was weaker when analysed for forest habitats ($r$ = 0.329; $P$ = 0.057).

High temperatures were associated with low relative humidity for the Fynbos ($r$ = -0.571, $P$ < 0.001) and forest ($r$ = -0.597, $P$ = 0.000) habitats across all study sites. Temperature and relative humidity were significantly different between the habitat types across all sites, as well as within each site (Table 3.5). The soil parameters (i.e. pH, P, Mg, C and N) of forest sites were significantly higher than the Fynbos sites, based on the combined data set ($t$ values were from -990.6 to -3.936, $P$ < 0.001 in all cases; Figure 3.7A-E). Similar trends were found in the analyses of individual study sites, although significance did not occur in all cases (Figure 3.7A-E).

## Discussion

This study found 89 *Seira* species across both Fynbos and Southern Afrotemperate forest habitats, with 67 species in the former and 32 in the latter. The number of *Seira* species sampled within these two habitat types across the region represents a four-fold increase of the current number of described species in the published literature of this genus in the Western Cape (Coates, 1968), which highlights the necessity for updating the alpha taxonomy of *Seira*. Despite the remarkable increases in *Seira* species richness demonstrated by this study, it is likely that the observed species richness in the Fynbos was underestimated as evidenced by the rarefaction curves and richness estimator which did not reach asymptotes in several of the sampled Fynbos sites.

At both local and regional scales, the species richness of *Seira* fauna recorded in the Fynbos was generally significantly higher in comparison with the forest habitats. Earlier studies have consistently found that the Fynbos biome is more species-rich for a broad spectrum of invertebrate taxa than the other Cape biomes (e.g. forest, thicket and Karoo) (Wright & Samways, 1998; Procheş & Cowling, 2006; Pryke & Samways, 2008, 2009b, 2010). It is unclear why Fynbos habitats have greater *Seira* species richness than the forest habitats. One possibility is that the exceptional environmental and floristic gradients that define the Fynbos communities (Manders et al., 1992; Goldblatt, 1997; Goldblatt & Manning, 2002; Cowling & Pierce, 2004; Latimer et al., 2005) could have contributed to the radiation of *Seira* species by providing a greater variety of potential ecological niches and floristic resources than the forest communities. Previous studies in other systems have found that the Collembola species richness increased in response to increased plant species richness (Robson et al. 2009; Zeppelini et al., 2009; Sabais et al., 2011) at the landscape scale (Vanbergen et al., 2007) and environmental heterogeneity at the finer spatial scale (Nielsen et al., 2010), which might explain the higher richness found in Fynbos. The positive impacts of plant diversity could have been due to the increase in plant litter diversity and quality, which act as food resources and habitats for Collembola (Sabais et al., 2011). However, the link between the two may also be an indirect one. Furthermore, the number of species is known to be positively correlated with the spatial extent of a particular habitat (MacArthur & Wilson, 1967), and smaller habitats may have been unable to sustain a diverse group of species due to limited ecological and demographical availability. Consequently the local and regional *Seira* species richness in forest habitats may be lower than in Fynbos habitats, because forest in the Cape tends to be spatially fragmented and less extensive (Cowling, 1990).

As predicted, *Seira* were more abundant in forest than in Fynbos at both local and regional scales. The litter layers in forest habitats have a significantly higher relative humidity and lower temperature compared with conditions in the Fynbos habitats. In addition, the internal conditions of the forest are known to be more equable due to the multi-layered closed-canopy system and substantial litter layer (Bergh et al., 2014), which increase buffering against unfavourable external macroclimatic conditions (Mucina & Geldenhuys, 2006). Several studies have found that the abundance of Collembola was positively affected by the soil moisture (Raschmanová et al., 2008; Robson et al., 2009; Zeppelini et al., 2013) or relative humidity, while negatively correlated with increasing temperature of their habitat (Liu et al., 2012; Raschmanová et al., 2013). Although the forests in the CFR have been known as plant communities with the fewest species (Bergh et al., 2014), the surface layer of forest soil generally has a high nutritional content (Mucina & Geldenhuys, 2006). In accordance with earlier studies (Cowling & Holmes, 1992; Manders et al., 1992; Cowling et al., 1997), the overall soil chemical contents were found to be significantly higher in the forest than the Fynbos soils in this study. In addition, positive relationships have been found with edaphic chemical contents and the abundance of Collembola (Chagnon et al., 2000; Raschmanová et al., 2008). These data suggest that the higher abundances of *Seira* in forest may be due to more suitable abiotic conditions in those habitats.

Although Collembola generally show substantial sensitivity to desiccation (Choi et al., 2002; Kærsgaard et al., 2004; Chown et al., 2007), the *Seira* species occupying the Fynbos habitat must be physically well-adapted to subsist the local environmental conditions of low relative humidity and high temperature in order to establish and persist in this open canopy system with low litter production (Cowling et al., 1997). It has been suggested that the cuticle structures of epigaeic (surface-dwelling) and hemi-edaphic (litter-dwelling) species of Collembola have developed lower permeability for water than the euedaphic (soil-dwelling) species (Verhoef & Witteveen, 1980; Kærsgaard et al., 2004). Based on personal observation and the morphological characteristics of *Seira* (i.e. well-developed furca, long legs and antenna), their traits are consistent with litter- and surface-dwelling species (Ponge et al., 2006); and therefore it is possible for them to have developed low cuticle permeability against water loss, or shift in vertical distribution for ideal microclimates in response to the environmental fluctuation in Fynbos habitat. On the other hand, the cooler temperature and higher relative humidity that characterises the indigenous forest appeared to be more suitable to the ecological requirements of forest-inhabiting *Seira* species, as they may be less tolerant to the arid and hot conditions than the Fynbos-inhabiting species.

Exceptionally high levels of *Seira* species turnover were found among and within habitat types across study sites within the CFR, which produced compositionally distinct *Seira* assemblages. Furthermore, habitat type (i.e. Fynbos or forest) and study sites were identified as significant factors in influencing the species composition. This level of turnover among sites is surprising, because it is plausible to assume that the mobility of *Seira* species can be relatively high through surface dispersal or passive transportation based on their anatomical traits, as it has been suggested for other Collembola species with similar anatomical characters (Hopkin, 1997; Dunger et al., 2002; Ponge et al., 2006). However, the majority of the *Seira* species in the CFR appear to be highly habitat and site specific; perhaps as a result of their close associations with particular abiotic and/or biotic variables, as previously discussed. A total of only ten localised habitat generalist species and nine broadly distributed habitat specialist species, thus almost each habitat type and study site was characterised by its own subset of unique *Seira* fauna. In the CFR, distinct insect assemblages have been found to be associated with each biomes (Procheş & Cowling, 2007). Moreover, similar level of turnover rates have been shown for the monkey beetle fauna between sites, with suggestions of steep environmental gradients combined with low dispersal capability and/or habitat specialisation of monkey beetles. Notably, the *Seira* assemblage structure in Grootvadersbosch in the Langeberg region appears to comprise of some individuals from both western and eastern sections of the CFR (Figure 3.5). The presence of overlapping specimens in this study site is most likely due to its intermediate geographic location above the deeply incised Breede River Valley Basin between the two geographical sections of the CFR.

Additionally, the rates of *Seira* species turnover are easily comparable to the highest rates of Fynbos plant community turnover between sites (Kruger & Taylor 1979; Cowling et al., 1992, 1996). High levels of turnover were also observed between forest sites, suggesting that the high turnover rates are a characteristic of this Collembola genus in the Fynbos and Southern Afrotemperate forest of the CFR. While I would have expected lower turnover between Fynbos sites than that of the forest sites, because Fynbos communities tend to be more continuous than the forest communities, this was not found in the present study. Therefore, the higher regional *Seira* species richness of the Fynbos habitat appears to be the result of richer local species within individual study sites than the forests. Moreover, the high beta diversity in both Fynbos and forest assemblages appears to have contributed significantly to the regional species richness of *Seira* in the CFR.

*Conclusions*

The present study is one of the first to have examined the spatial diversity of a soil invertebrate group in the CFR, and in particular to quantify beta diversity, which appears astonishingly high for *Seira*. The composition of *Seira* species was almost completely different between Fynbos and Southern Afrotemperate forest habitats, as well as among most study sites of the same habitat type. This chapter sampled only two of South Africa's eight biomes for *Seira*, and within the Fynbos biome only a fraction of the available habitat was sampled. Given the high degree of habitat fidelity and localisation of most *Seira* species, it is likely that the remaining available areas harbour many undiscovered species. I predict that the 89 *Seira* species uncovered in this chapter is but a portion of the species present in the CFR.

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**Table 3.1.** The total number and abundance of species from the genus *Seira* collected in the Fynbos and Southern Afrotemperate forest of different study sites. Bold letters indicate study sites with cryptic species.

| Site | Habitat | Total species richness | Total shared species | Total abundance |
|---|---|---|---|---|
| **Combined** | Fynbos | 57 | 10 | 2685 |
| | Southern Afrotemperate forest | 22 | | 2997 |
| Groot Wintershoek | Fynbos | 3 | / | 22 |
| Table Mountain National Park | Fynbos | 9 | 2 | 286 |
| | Southern Afrotemperate forest | 4 | | 492 |
| **Helderberg** | Fynbos | 7 | 0 | 462 |
| | Southern Afrotemperate forest | 3 | | 149 |
| Kogelberg | Fynbos | 12 | 1 | 396 |
| | Southern Afrotemperate forest | 5 | | 255 |
| **De Hoop Nature Reserve** | Fynbos | 13 | / | 591 |
| **Grootvadersbosch Nature Reserve** | Fynbos | 11 | 3 | 307 |
| | Southern Afrotemperate forest | 7 | | 990 |
| **Outeniqua Nature Reserve** | Fynbos | 6 | 3 | 358 |
| | Southern Afrotemperate forest | 7 | | 366 |
| **Tsitsikamma National Park** | Fynbos | 6 | 0 | 117 |
| | Southern Afrotemperate forest | 2 | | 203 |
| Baviaanskloof | Fynbos | 10 | 1 | 146 |
| | Southern Afrotemperate forest | 7 | | 542 |

**Table 3.2.** Differences in the species richness per sampling patch of *Seira* between Fynbos and Southern Afrotemperate forest determined using GLMMs (all study sites combined) and GLMs (individual study site). Bold indicates sites with cryptic species. Red indicates significant *P* value.

| | Mean ± SE | | *z* value | *P* value |
|---|---|---|---|---|
| | Fynbos | Southern Afrotemperate forest | | |
| Combined (excluding Groot Wintershoek and De Hoop) | 4.857 ± 0.348 | 2.679 ± 0.236 | 4.148 | 0.000 |
| Groot Wintershoek | 1.5 ± 0.645 | / | / | / |
| Table Mountain National Park | 5.75 ± 0.629 | 2.25 ± 0.479 | 2.386 | 0.017 |
| **Helderberg** | 5 ± 0.577 | 2 ± 0.408 | 2.19 | 0.028 |
| Kogelberg | 4.5 ± 0.866 | 2.75 ± 0.629 | 1.287 | 0.198 |
| **De Hoop Nature Reserve** | 7 ± 1.291 | / | / | / |
| **Grootvaderbosch Nature Reserve** | 7.75 ± 0.479 | 4.25 ± 0.854 | 1.991 | 0.047 |
| **Outeniqua Nature Reserve** | 4 ± 0.707 | 3.75 ± 0.25 | 0.18 | 0.857 |
| **Tsitsikamma National Park** | 3.5 ± 0.645 | 2 ± 0 | 1.263 | 0.207 |
| Baviaanskloof | 3.5 ± 0.645 | 1.75 ± 0.25 | 1.497 | 0.134 |

**Table 3.3.** Differences in the abundance per sampling patch of *Seira* between Fynbos and Southern Afrotemperate forest determined using the GLMM (all study sites combined) and GLMs (individual study site). Red indicates significant *P* value.

| | Mean ± SE | | *t* value | *P* value |
|---|---|---|---|---|
| | Fynbos | Southern Afrotemperate forest | | |
| Combined (excluding Groot Wintershoek and De Hoop) | 74 ± 13.730 | 107.036 ± 17.732 | -1.505 | 0.132 |
| Groot Wintershoek | 5.500 ± 2.754 | / | / | / |
| Table Mountain National Park | 71.500 ± 13.555 | 123 ± 18.713 | -2.217 | 0.069 |
| Helderberg | 115.500 ± 83.499 | 37.250 ± 21.437 | 0.996 | 0.358 |
| Kogelberg | 99 ± 41.649 | 63.750 ± 29.398 | 0.695 | 0.513 |
| De Hoop Nature Reserve | 147.750 ± 55.362 | / | / | / |
| Grootvaderbosch Nature Reserve | 76.750 ± 11.272 | 247.500 ± 41.031 | -4.358 | 0.005 |
| Outeniqua Nature Reserve | 89.500 ± 26.509 | 91.500 ± 34.345 | -0.046 | 0.965 |
| Tsitsikamma National Park | 29.250 ± 7.087 | 50.750 ± 5.234 | -2.234 | 0.067 |
| Baviaanskloof | 36.500 ± 10.087 | 135.500 ± 70.201 | -1.590 | 0.163 |

**Table 3.4.** Multisite dissimilarities for the total beta diversity (Sørensen's index of dissimilarity, $\beta_{SOR}$), turnover- (Simpson dissimilarity, $\beta_{SIM}$) and nestedness-resultant dissimilarity ($\beta_{NES}$) for the *Seira* assemblages in the Fynbos and Southern Afrotemperate forest habitat across all study sites. Groot Wintershoek and De Hoop were excluded from the analyses because the forest was not represented in those sites.

| | Both habitat types combined | Fynbos | Southern Afrotemperate forest |
|---|---|---|---|
| $\beta_{SOR}$ | 0.968 | 0.962 | 0.971 |
| $\beta_{SIM}$ | 0.957 | 0.954 | 0.962 |
| $\beta_{SNE}$ | 0.011 | 0.008 | 0.010 |

**Table 3.5.** Comparison of the temperature and relative humidity between Fynbos and Southern Afrotemperate forest using the GLMMs (all study sites combined) and GLMs (individual study site), assuming gamma distribution with identity function. Red indicates significant *P* value.

| | Temperature (˚C) | | | | Relative humidity (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean ± SE | | *t* value | *P* value | Mean ± SE | | *t* value | *P* value |
| | Fynbos | Southern Afrotemperate forest | | | Fynbos | Southern Afrotemperate forest | | |
| Combined (excluding Groot Wintershoek and De Hoop) | 27.126 ± 3.881 | 21.909 ± 0.211 | -21.230 | < 0.001 | 53.013 ± 0.775 | 71.156 ± 0.680 | 21.640 | < 0.001 |
| Groot Wintershoek | 15.227 ± 2.310 | / | / | / | 75.390 ± 10.061 | / | / | / |
| Table Mountain National Park | 26.773 ± 0.487 | 22.100 ± 0.380 | -7.135 | < 0.001 | 42.198 ± 1.365 | 58.065 ± 1.242 | 8.060 | < 0.001 |
| Helderberg | 23.385 ± 4.666 | 18.895 ± 0.143 | -6.728 | < 0.001 | 57.650 ± 2.197 | 74.712 ± 1.144 | 5.867 | < 0.001 |
| Kogelberg | 26.625 ± 0.436 | 20.644 ± 0.486 | -8.743 | < 0.001 | 46.798 ± 1.836 | 73.333 ± 1.752 | 8.405 | < 0.001 |
| De Hoop Nature Reserve | 28.113 ± 0.370 | / | / | / | 50.735 ± 1.002 | / | / | / |
| Grootvaderbosch Nature Reserve | 29.477 ± 0.498 | 19.200 ± 0.156 | -22.040 | < 0.001 | 64.233 ± 1.513 | 85.663 ± 0.763 | 11.240 | < 0.001 |
| Outeniqua Nature Reserve | 29.215 ± 0.450 | 25.235 ± 0.692 | -4.635 | < 0.001 | 60.396 ± 1.140 | 72.144 ± 1.880 | 5.488 | < 0.001 |
| Tsitsikamma National Park | 28.046 ± 0.434 | 25.377 ± 0.488 | -4.045 | < 0.001 | 61.356 ± 1.210 | 71.293 ± 1.253 | 5.654 | < 0.001 |
| Baviaanskloof | 26.660 ± 0.413 | 20.931 ± 0.310 | -11.160 | < 0.001 | 38.456 ± 1.121 | 63.871 ± 1.077 | 14.310 | < 0.001 |

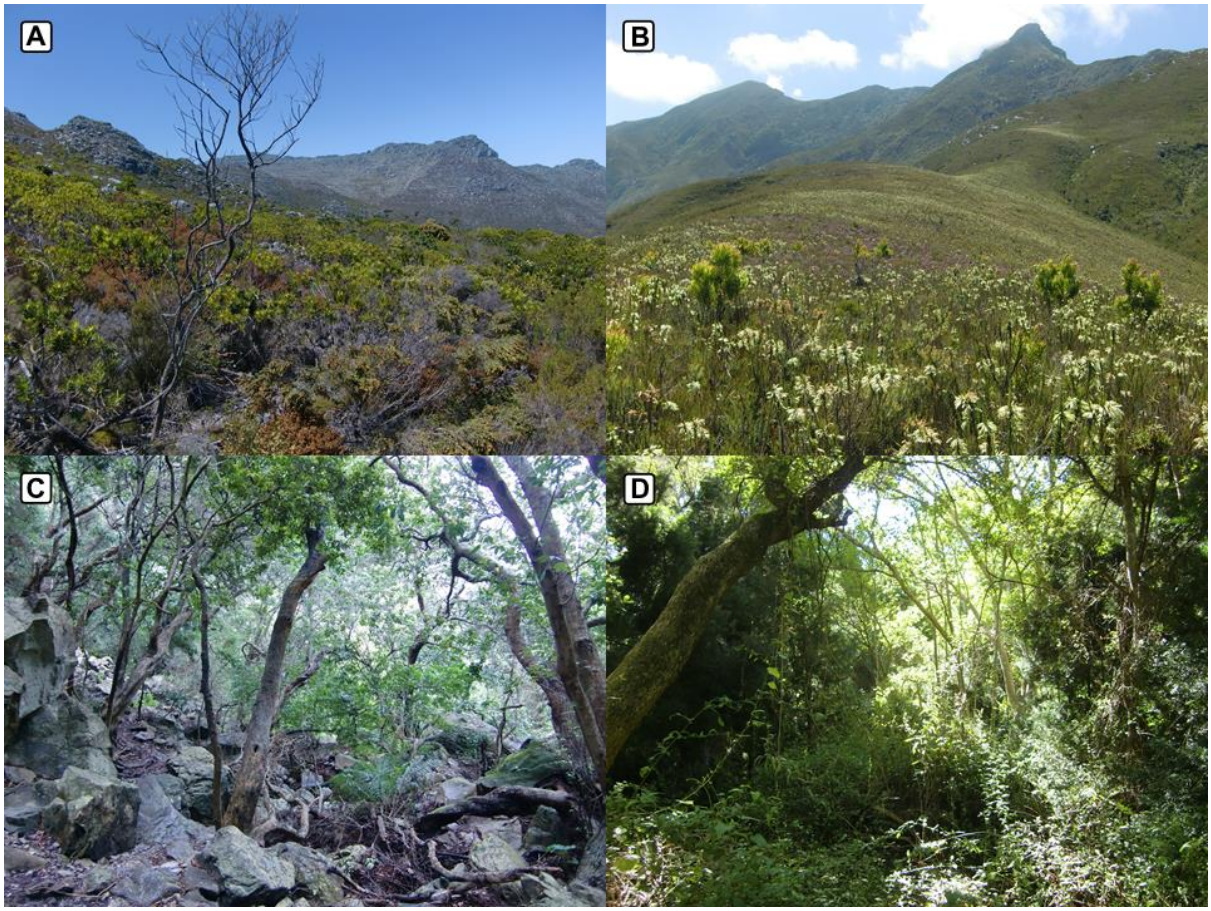


**Figure 3.1.** Examples of Fynbos and Southern Afrotemperate forest habitats. (A) Silvermine Nature Reserve in Table Mountain National Park: Peninsula Sandstone Fynbos. (B) Outenqiua Nature Reserve: South Outeniqua Sandstone Fynbos. The Southern Afrotemperate forest habitats in (C) Harold Porter National Botanical Garden (forms part of the Kogelberg samples), and (D) Geelhoutbos in Baviaanskloof.

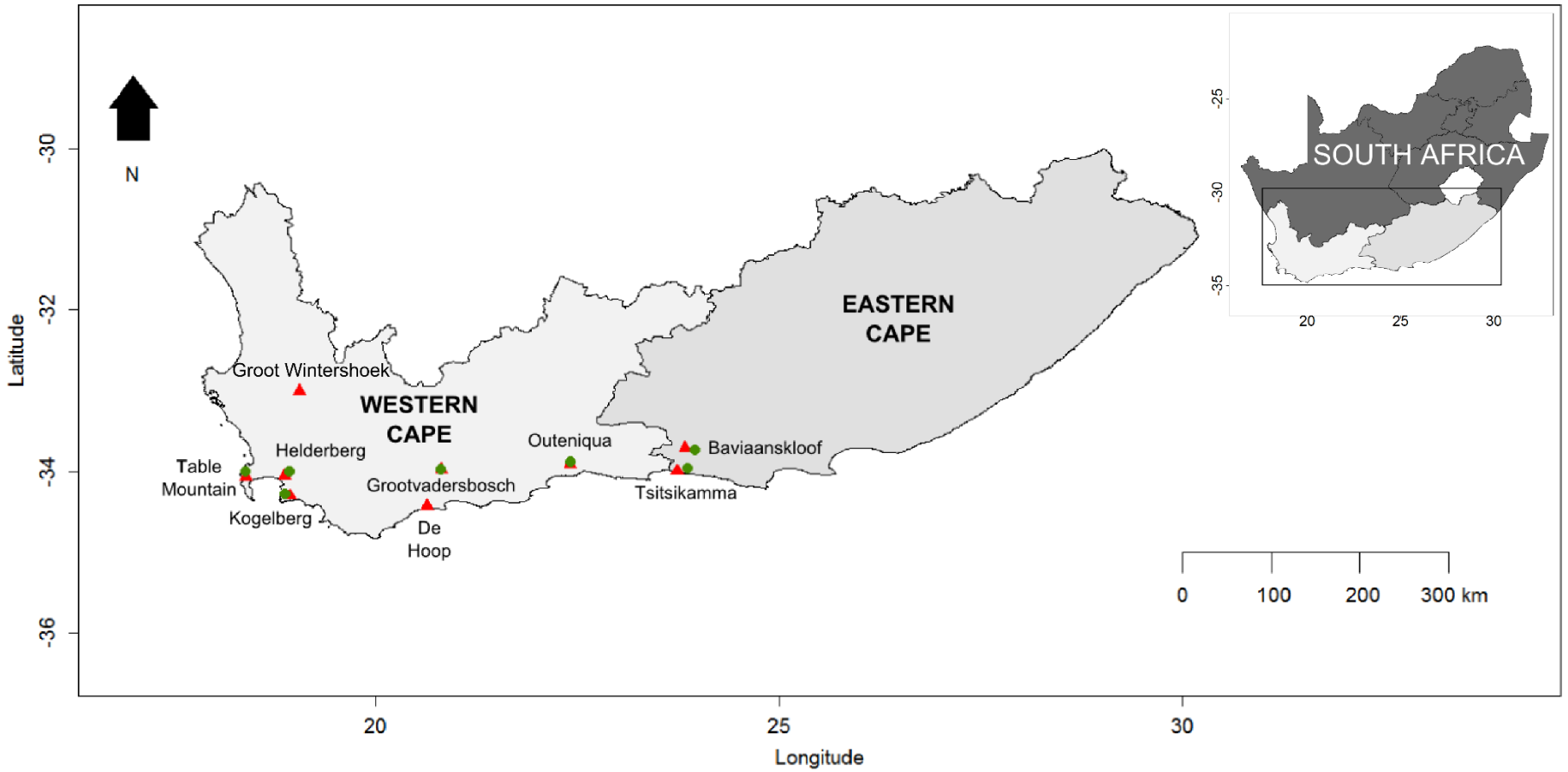


**Figure 3.2.** Locations of the nine study sites in the Cape Floristic Region (CFR). The Fynbos (▲) and Southern Afrotemperate forest (●) habitats are marked within seven of the sampled sites in the two provinces. Groot Wintershoek and De Hoop were only represented by the Fynbos habitat.

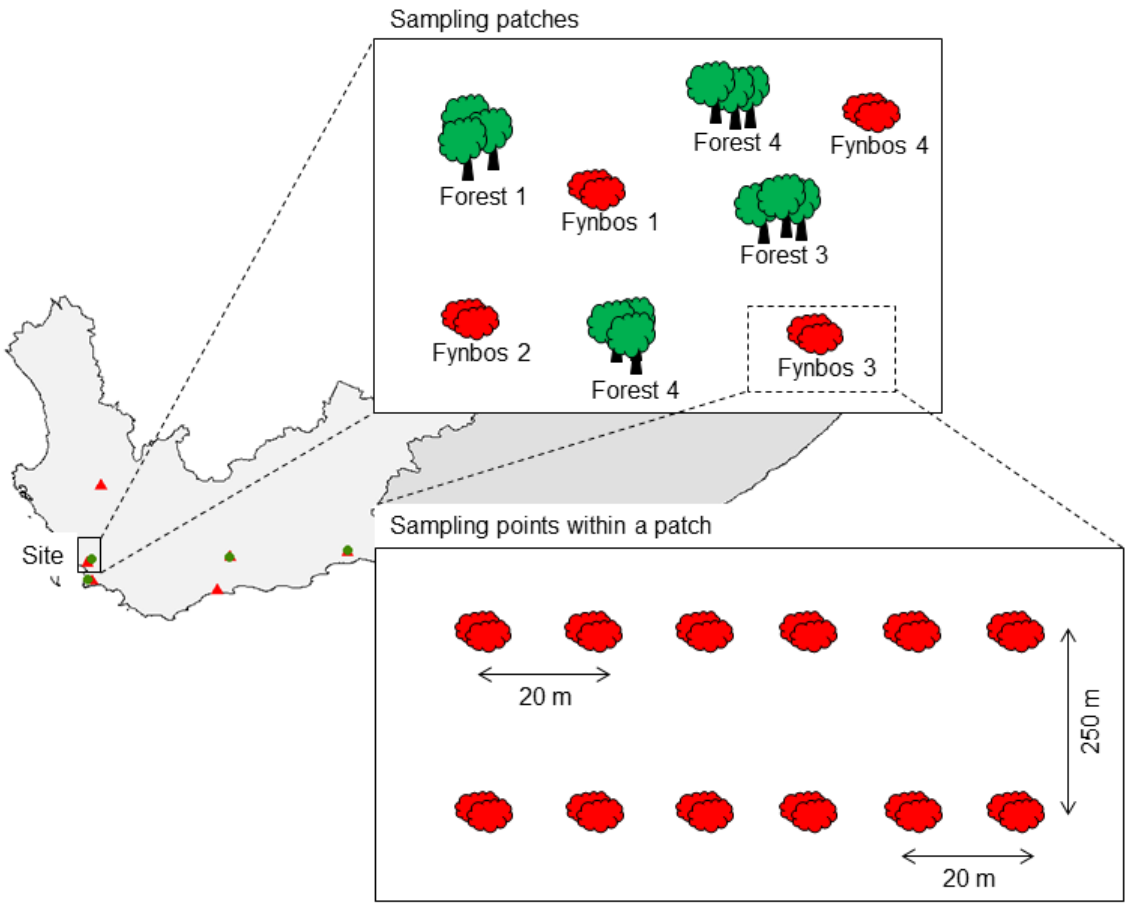


**Figure 3.3.** An illustration of the sampling method employed in the Fynbos (red) and Southern Afrotemperate forest (green) patches within the study sites.

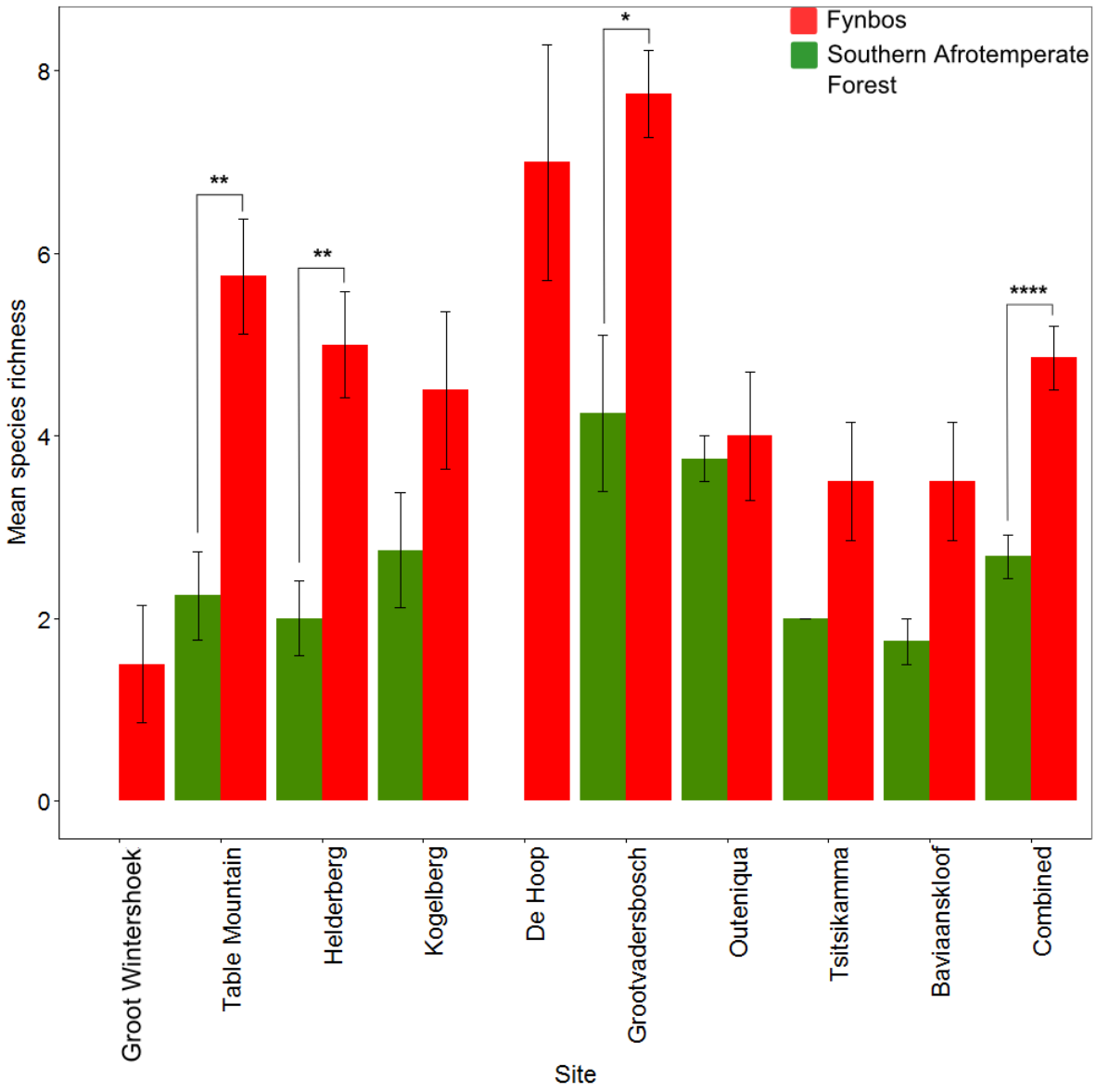


**Figure 3.4A.** Mean species richness of *Seira* in the Fynbos and Southern Afrotemperate forest per study site, as well as all sites combined (excluding Groot Wintershoek and De Hoop). GLMMs and GLMs with Poisson error distribution assumption with logarithmic link were used to test the significance among habitat types. $^{*}P = 0.050$, $^{**}P < 0.050$, $^{***}P < 0.010$, $^{****}P < 0.001$. The whiskers indicate standard errors.

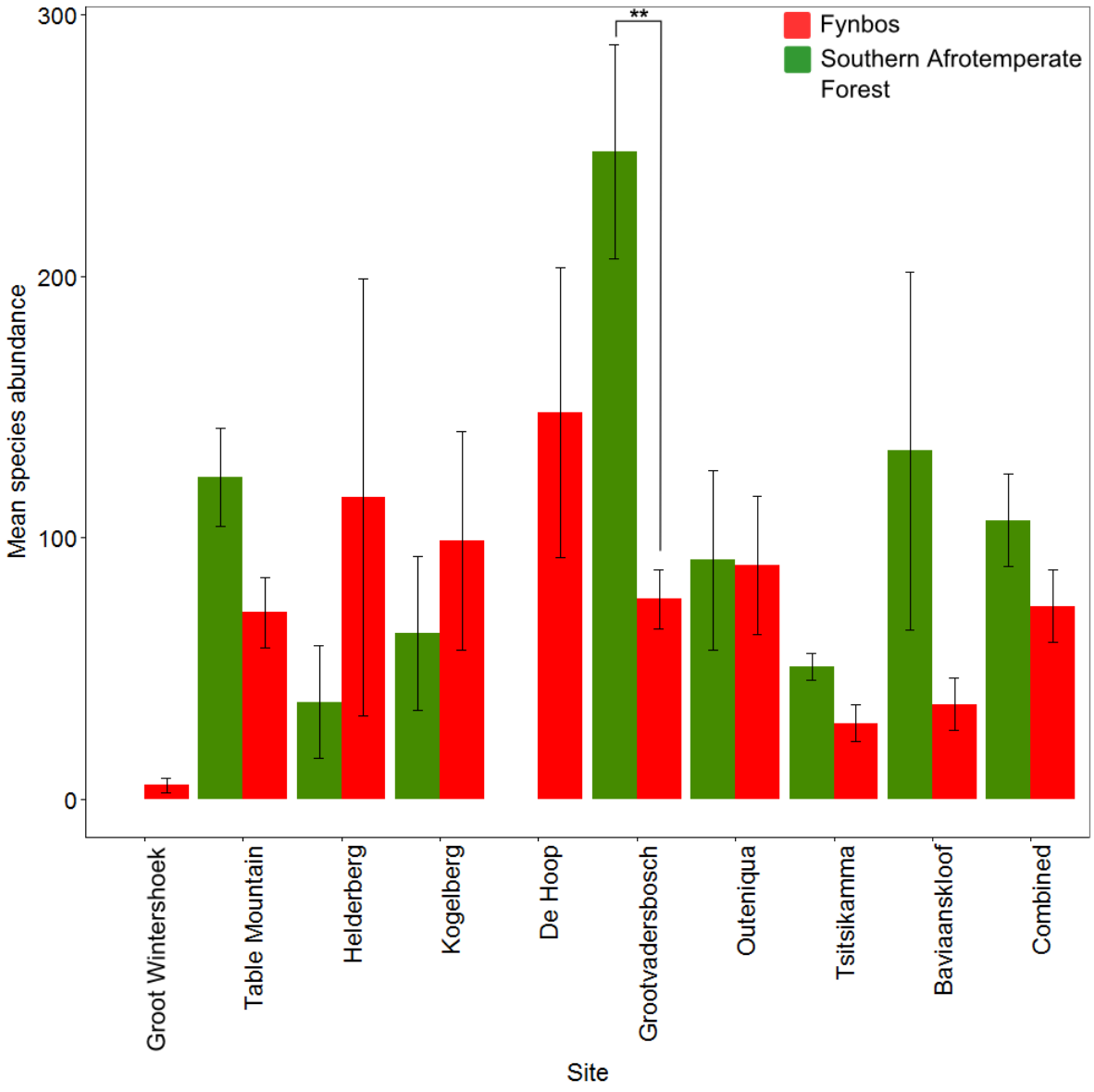


**Figure 3.4B.** Mean abundance of *Seira* in the Fynbos and Southern Afrotemperate forest per study site, as well as all sites combined (excluding Groot Wintershoek and De Hoop). GLMMs and GLMs with Negative Binomial error distribution to account for the overdispersion were used to test the significance among habitat types. $^{*}P = 0.050$, $^{**}P < 0.050$, $^{***}P < 0.010$, $^{****}P < 0.001$. The whiskers indicate standard errors.

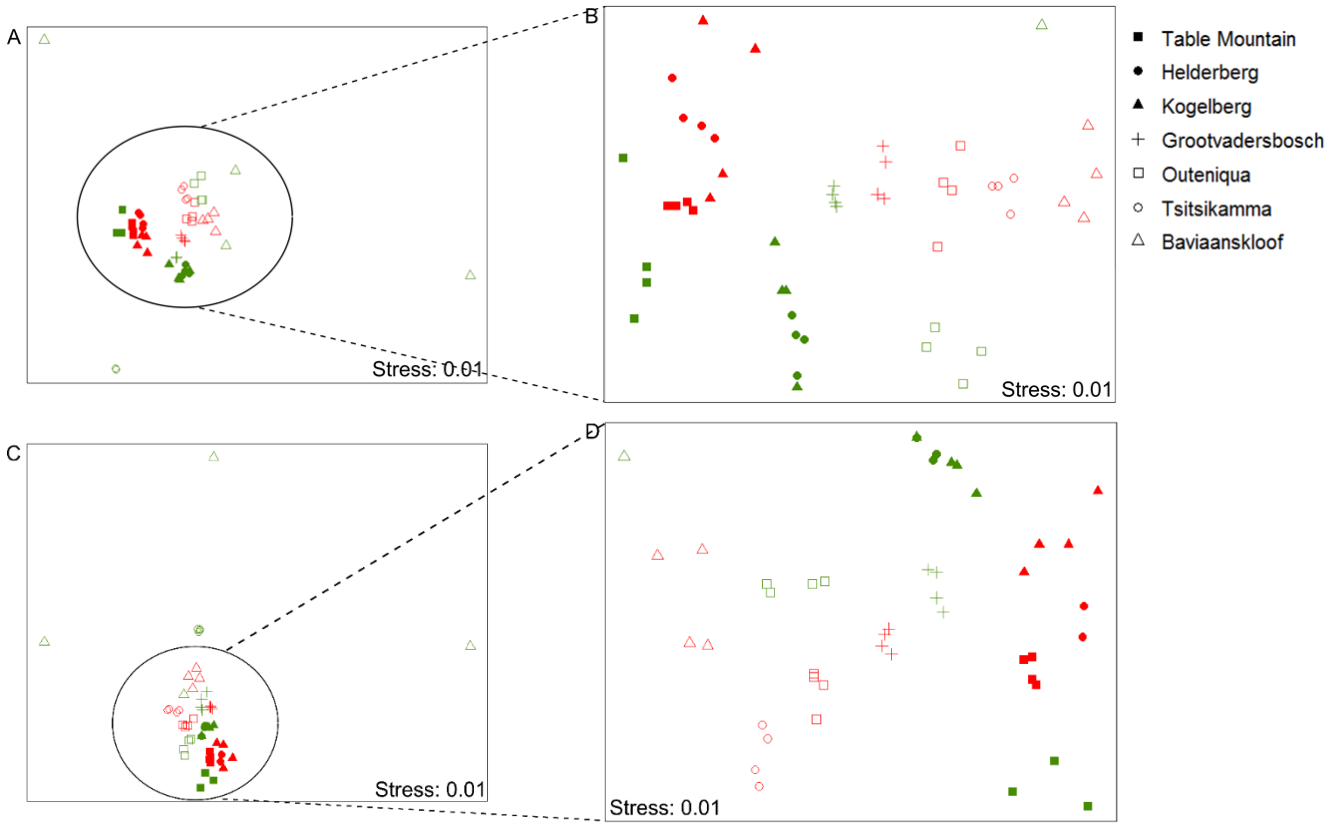


**Figure 3.5.** The *Seira* assemblage structures of the habitat patches nested in each of the study sites based on the (A) species abundance with outliers; among habitat types ANOSIM Global $R$ = 0.838, $P$ = 0.001; among study sites ANOSIM Global $R$ = 0.588, $P$ = 0.001, (B) species abundance without outliers; among habitat types ANOSIM Global $R$ = 0.8889, $P$ = 0.001; among study sites ANOSIM Global $R$ = 0.692, $P$ = 0.001, (C) species identity with outliers; among habitat types ANOSIM Global $R$ = 0.842, $P$ = 0.001; among study sites ANOSIM Global $R$ = 0.599, $P$ = 0.001, and (D) species identity without outliers; among habitat types ANOSIM Global $R$ = 0.882, $P$ = 0.001; among study sites ANOSIM Global $R$ = 0.701, $P$ = 0.001. Each point represents a habitat patch within the study sites (Fynbos: $n$ = 4; forest: $n$ = 4); different shapes correspond to different study sites; red represents Fynbos; green represents Southern Afrotemperate forest.

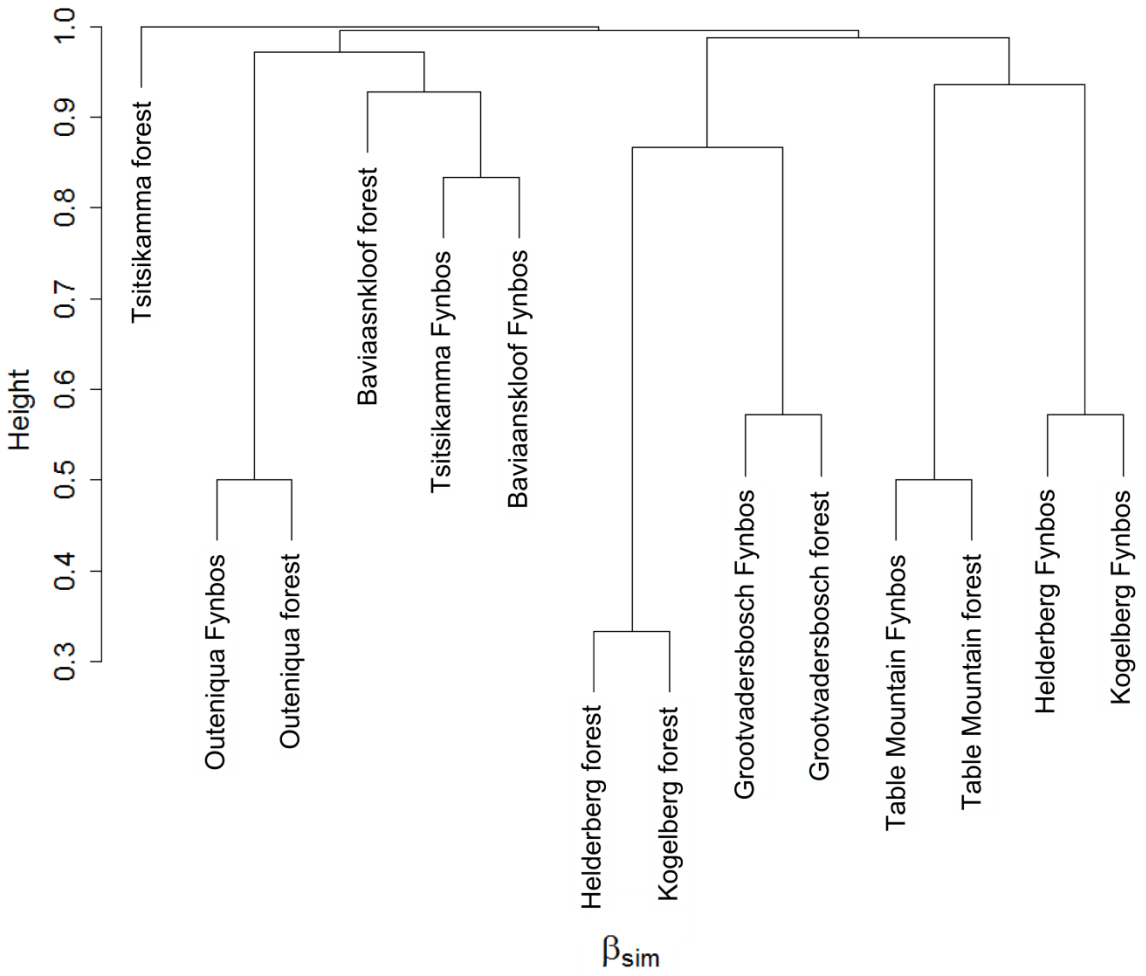


**Figure 3.6A.** Spatial turnover ($\beta_{sim}$) of *Seira* species composition across study sites from either Fynbos or Southern Afrotemperate forest habitats. Dissimilarity increases with branch length.

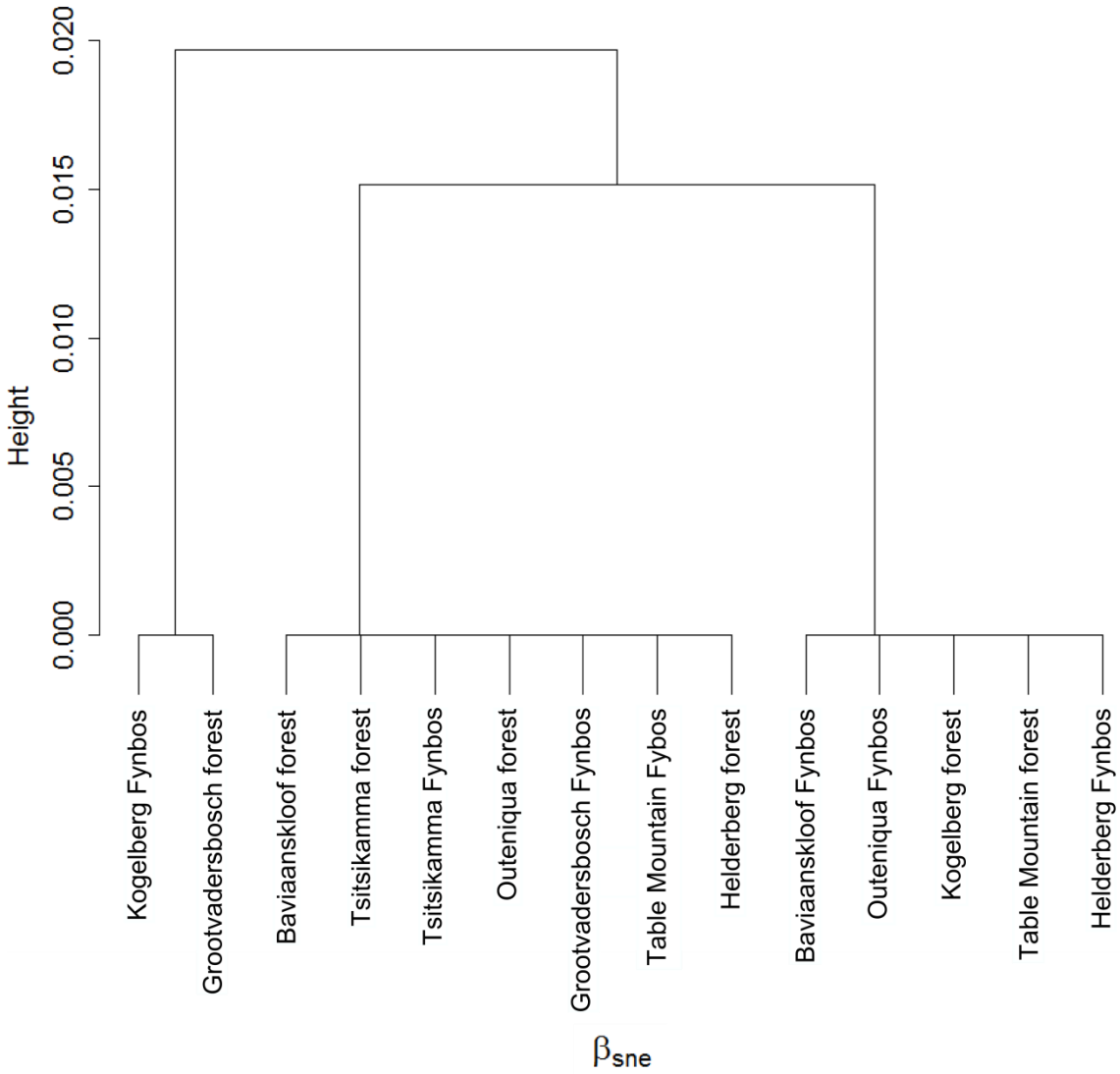


**Figure 3.6B.** Nestedness ($\beta_{sne}$) of *Seira* species composition across study sites from either Fynbos or Southern Afrotemperate forest habitats. Dissimilarity increases with branch length.

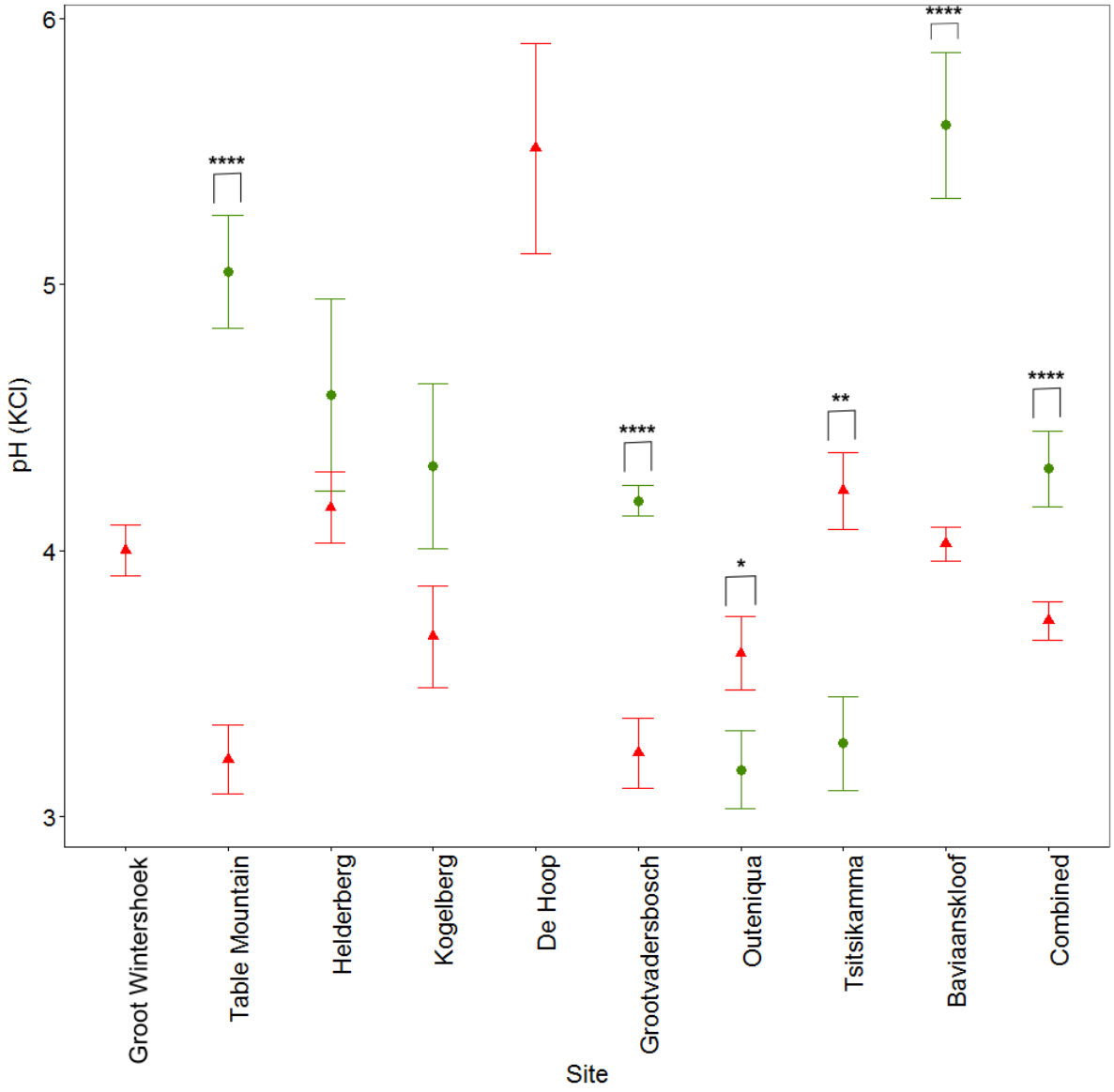


**Figure 3.7A.** Mean soil pH in the Fynbos and Southern Afrotemperate forest per study site, as well as all sites combined (excluding Groot Wintershoek and De Hoop). $^{*}P = 0.050$, $^{**}P < 0.050$, $^{***}P < 0.010$, $^{****}P < 0.001$. Whiskers indicate standard error; red triangle represents Fynbos and green circle represents Southern Afrotemperate forest.

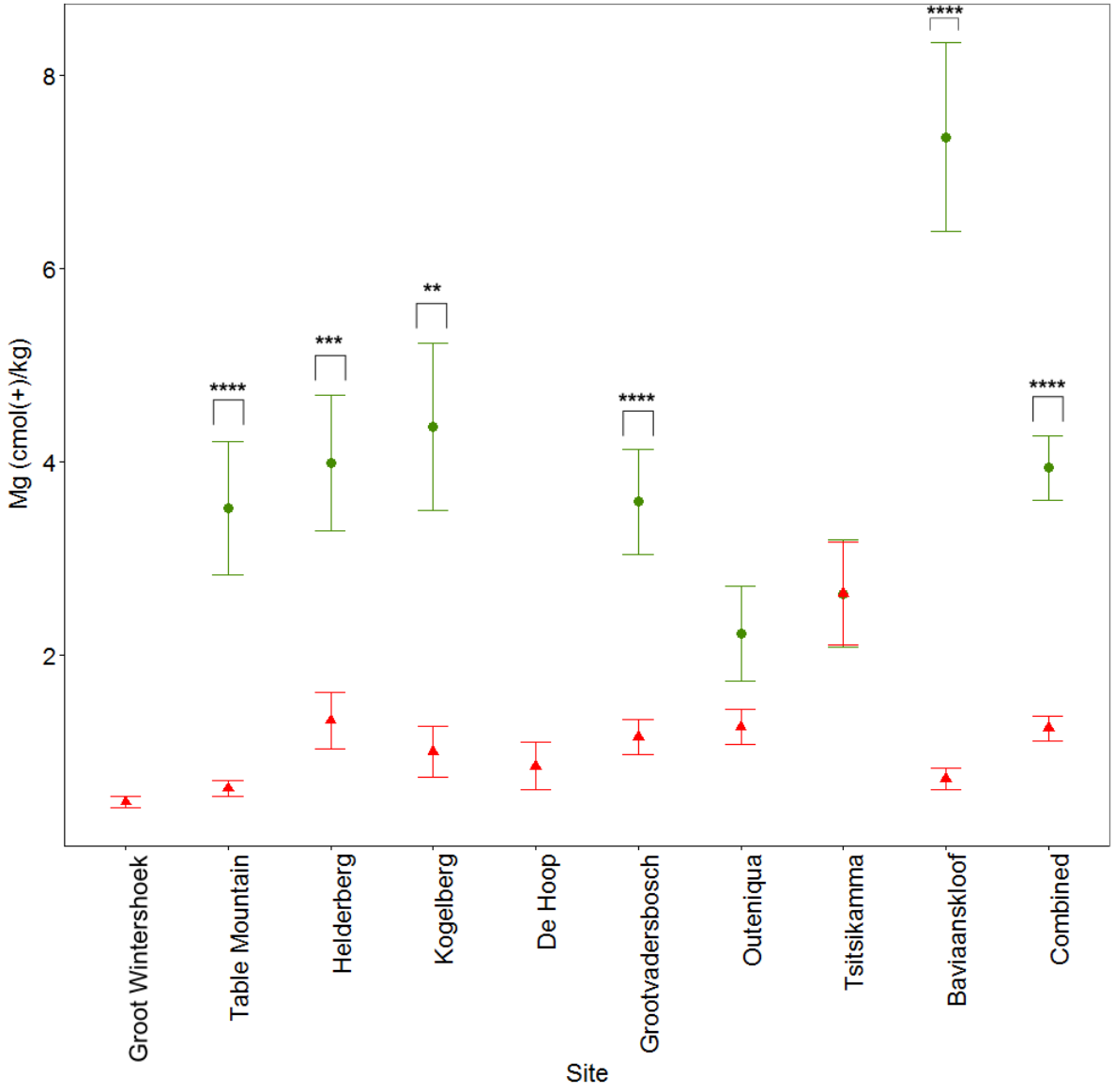


**Figure 3.7B.** Mean soil Magnesium (Mg) in the Fynbos and Southern Afrotemperate forest per study site, as well as all sites combined (excluding Groot Wintershoek and De Hoop). $^{*}P = 0.050$, $^{**}P < 0.050$, $^{***}P < 0.010$, $^{****}P < 0.001$. Whiskers indicate standard errors; red triangles represent Fynbos and green circles represent Southern Afrotemperate forest.

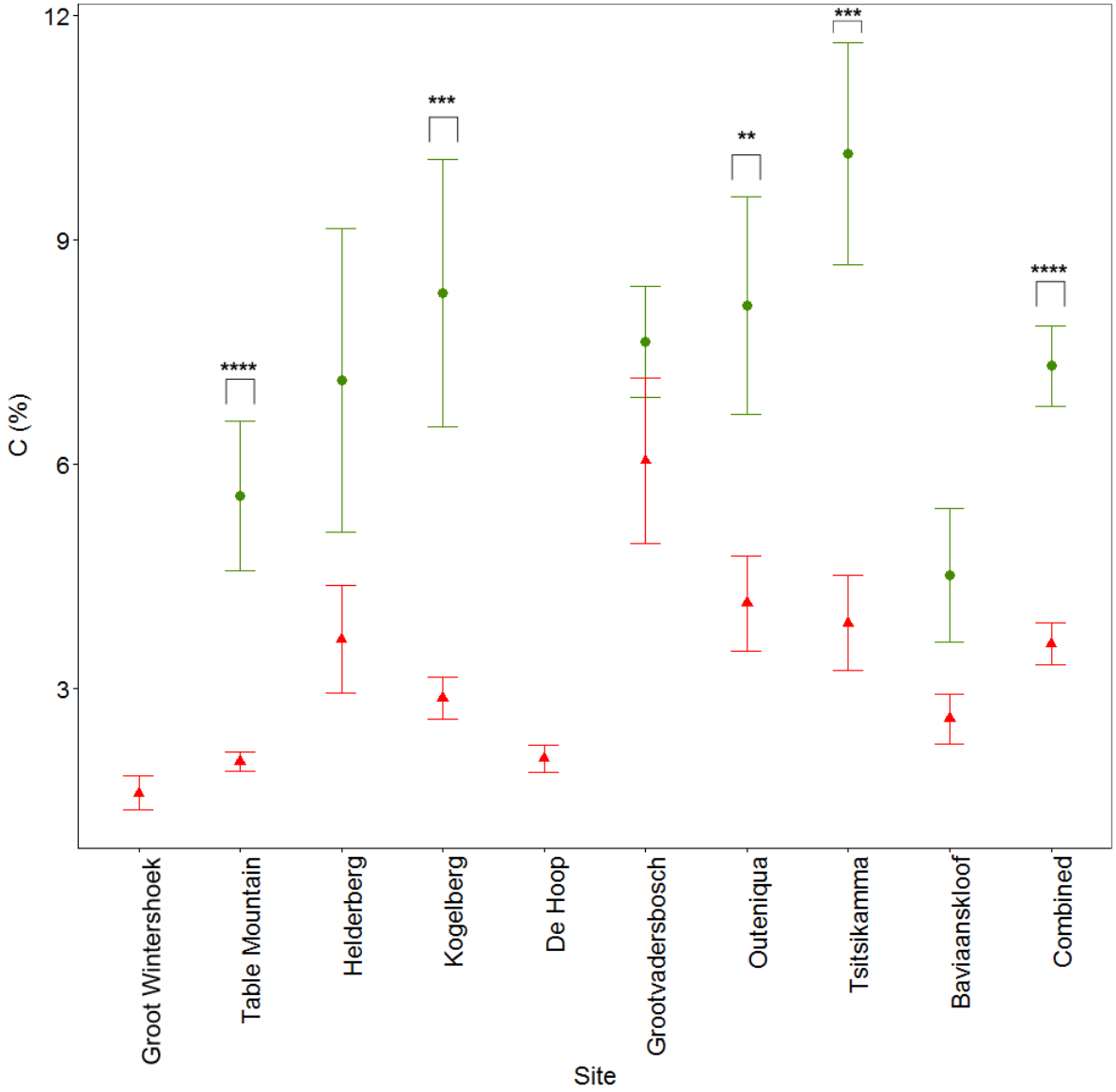


**Figure 3.7C.** Mean soil Carbon (C) in the Fynbos and Southern Afrotemperate forest per study site, as well as all sites combined (excluding Groot Wintershoek and De Hoop). $^{*}P = 0.050$, $^{**}P < 0.050$, $^{***}P < 0.010$, $^{****}P < 0.001$. Whiskers indicate standard errors; red triangles represent Fynbos and green circles represent Southern Afrotemperate forest.

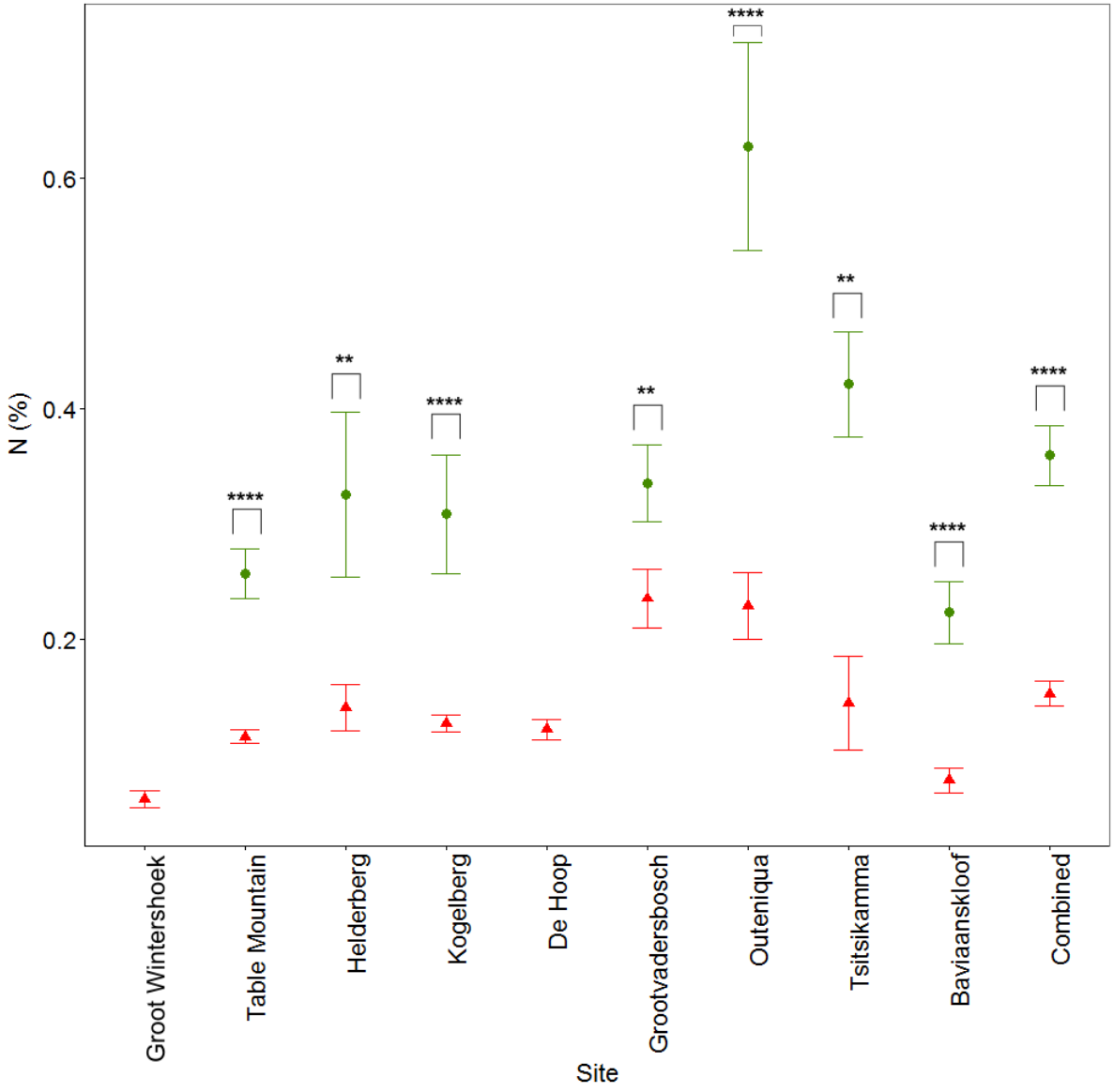


**Figure 3.7D.** Mean soil Nitrogen (N) in the Fynbos and Southern Afrotemperate forest per study site, as well as all sites combined (excluding Groot Wintershoek and De Hoop). $^{*}P$ = 0.050, $^{**}P < 0.050$, $^{***}P < 0.010$, $^{****}P < 0.001$. Whiskers indicate standard errors; red triangles represent Fynbos and green circles represent Southern Afrotemperate forest.

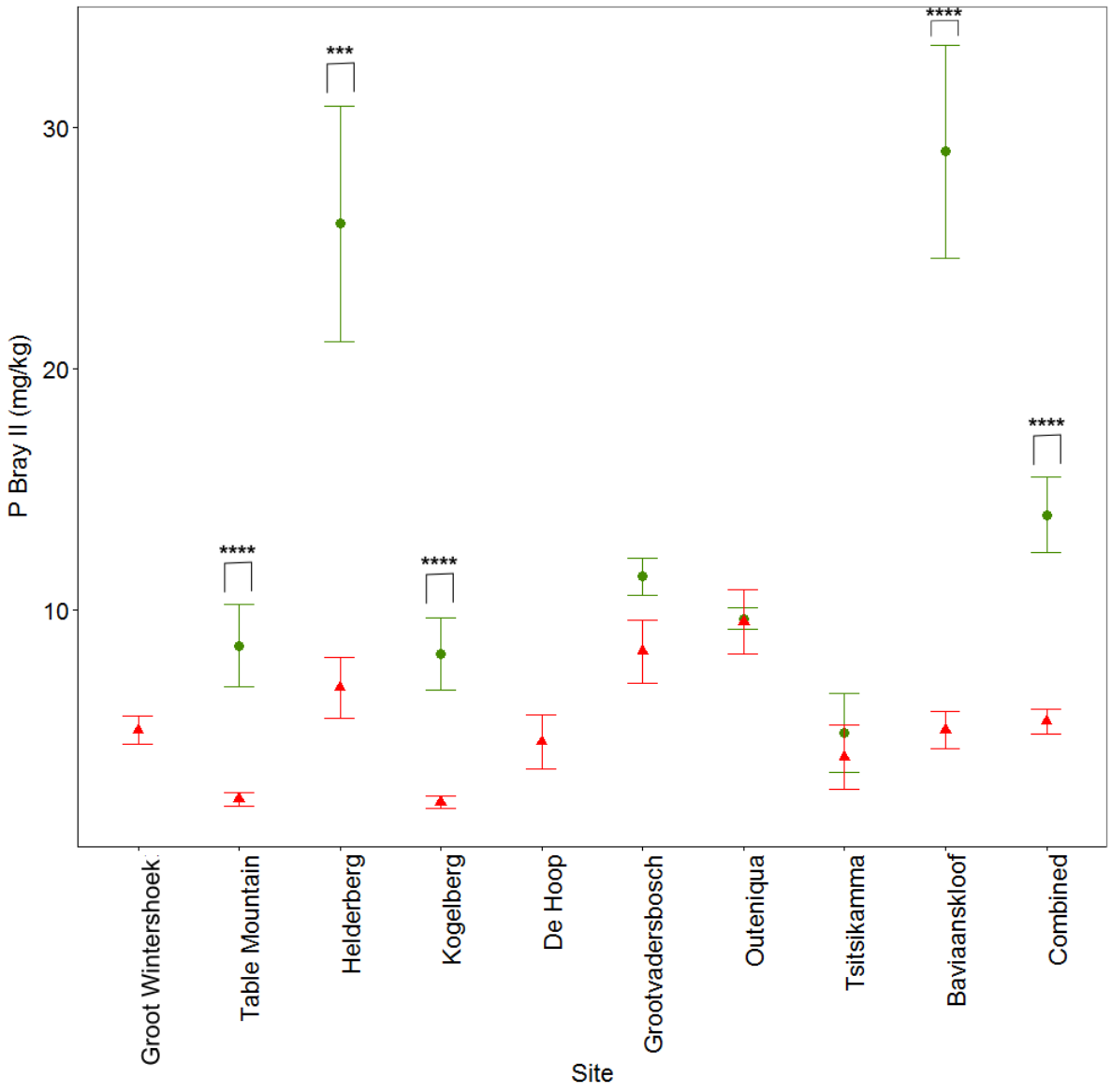


**Figure 3.7E.** Mean soil Phosphorus (P Bray II) in the Fynbos and Southern Afrotemperate forest per study site, as well as all sites combined (excluding Groot Wintershoek and De Hoop). $^{*}P = 0.050$, $^{**}P < 0.050$, $^{***}P < 0.010$, $^{****}P < 0.001$. Whiskers indicate standard error; red triangle represents Fynbos and green circle represents Southern Afrotemperate forest.

## Supplementary Figures

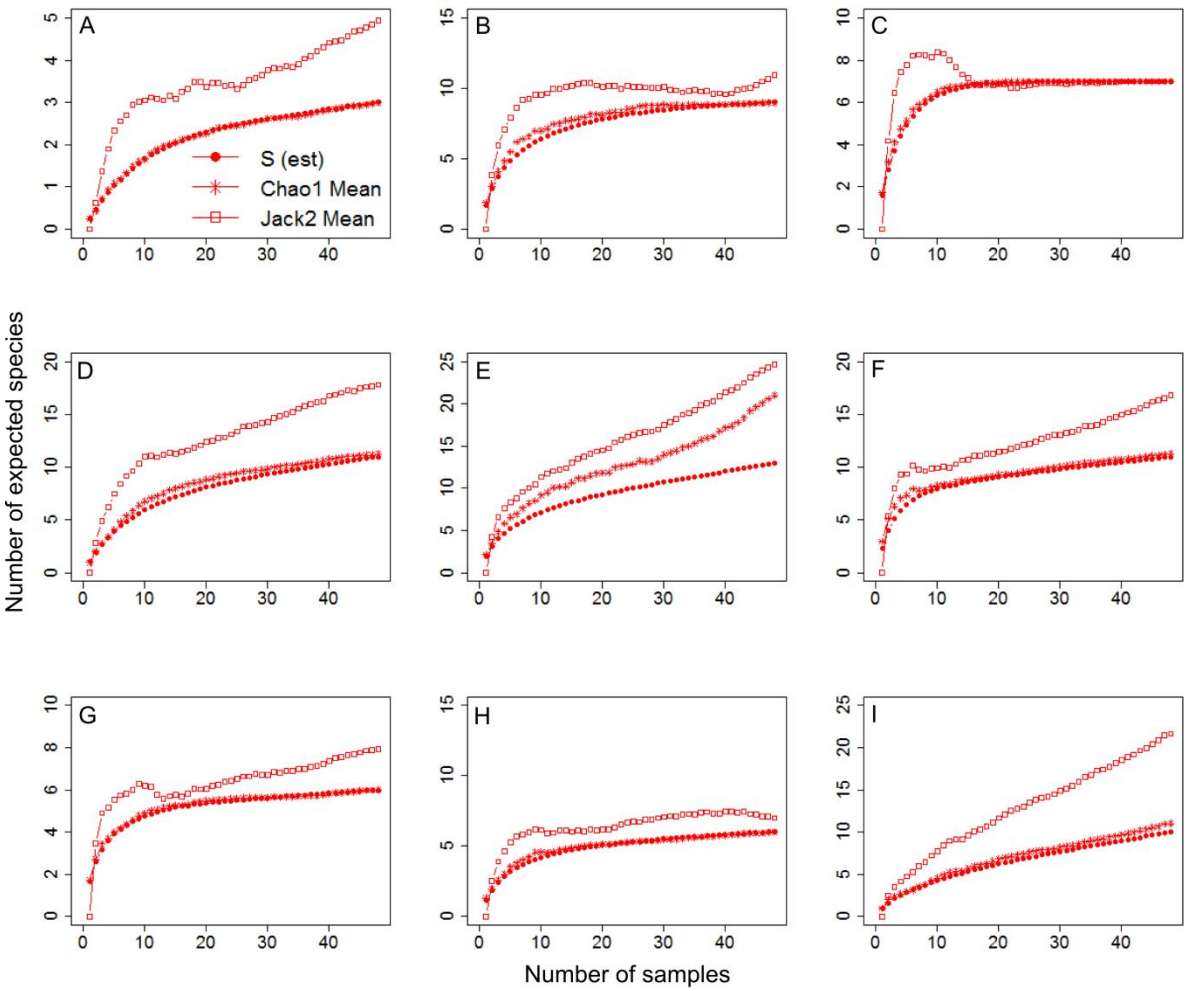


**Figure S3.1.** Sample-based rarefaction curves of *Seira* assemblages (including cryptic species) in the Fynbos habitat of each study site. (A) Groot Wintershoek, (B) Table Mountain National Park, (C) Helderberg, (D) Kogelberg, (E) De Hoop Nature Reserve, (F) Grootvadersbosch Nature Reserve, (G) Outeniqua Nature Reserve, (H) Tsitsikamma National Park, and (I) Baviaanskloof. The number of expected species in the pooled samples (S est); Chao 1 richness estimator (Chao1 mean); Second-order Jackknife richness estimator (Jack2 Mean).

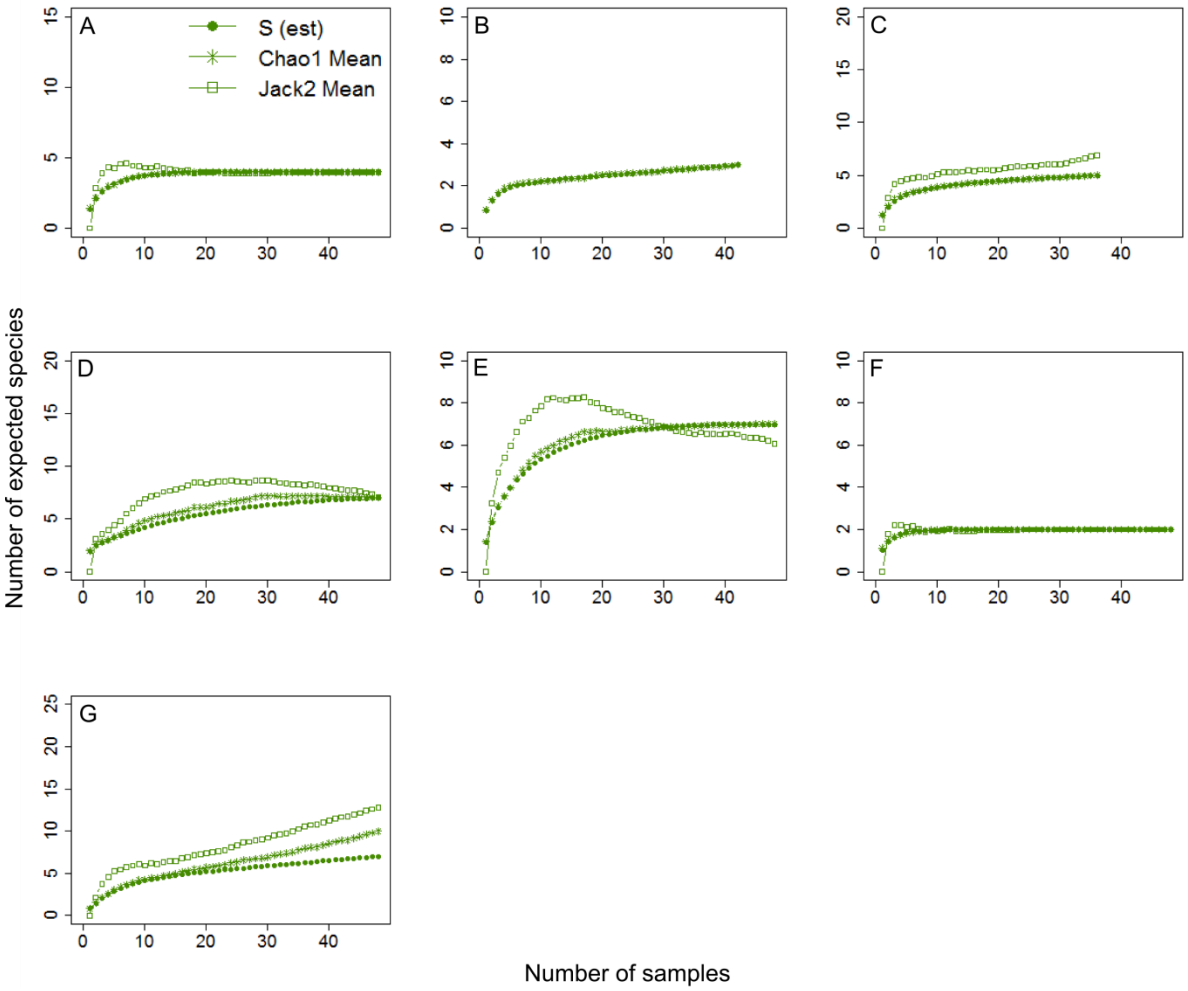


**Figure S3.2.** Sample-based rarefaction curves of *Seira* assemblages in the Southern Afrotemperate forest habitat of each study locality. (A) Table Mountain National Park, (B) Helderberg, (C) Kogelberg, (D) Grootvadersbosch Nature Reserve, (E) Outeniqua Nature Reserve, (F) Tsitsikamma National Park, and (G) Baviaanskloof. The number of expected species in the pooled samples (S est); Chao 1 richness estimator (Chao1 mean); Second-order Jackknife richness estimator (Jack2 Mean).

## Chapter 4

## General Conclusion

Soil is recognised as one of the most complex and understudied terrestrial ecosystems (Decaëns, 2010), and when compared with the aboveground biota, our understanding of its diverse fauna is limited in terms of both taxonomical and ecological perspectives (Bardgett & van der Putten, 2014). This is particularly true for the Cape Floristic Region (CFR), with marked bias in research towards higher plants (e.g. Cowling et al., 1992; Goldblatt & Manning, 2002; Linder, 2003) and pollinating invertebrates (e.g. Johnson, 1992; Colville, 2009; Braschler et al., 2012). Despite their diversity and functional significance in the delivery of ecosystem services that directly and indirectly impact human welfare, soil fauna have been particularly underrepresented in the formulation of global conservation initiatives and policies (Hågvar, 1998; Decaëns et al., 2006). Therefore, there is an increasing need to obtain baseline data that are applicable in the monitoring and conservation of soil biodiversity, in an effort to reduce the rate of biodiversity loss and ensure the continuation of terrestrial ecosystem integrity and human welfare.

The main aim of this thesis was to investigate the diversity of one of the more diverse Collembola genera, *Seira*, in the Fynbos and Southern Afrotemperate forest habitats of the CFR, based on the mitochondrial cytochrome oxidase subunit I (COI) DNA barcode. The results revealed unexpectedly high numbers of Molecular Operational Taxonomic Units (MOTUs) within the genus *Seira*. This indicates the potential existence of numerous previously overlooked or newly encountered species in this region (Chapter two), known better for its unusually high plant species richness. The number of discovered *Seira* MOTUs in both chapter two and three well exceeded the number of species described by the alpha taxonomic studies in the Western Cape (i.e. four-fold increase). These findings suggest that the underlying diversity within this group is much higher than previously thought based on our current taxonomic knowledge and the number of morphospecies. Furthermore, the species richness in the Fynbos habitat appears to be significantly higher than in the forest at both local and regional scale (Chapter three). Further work should focus on the identification and description of these discovered *Seira* MOTUs at high taxonomic resolution; which can be achieved through comprehensive taxonomic analysis by combining detailed morphological and ecological data, as well as additional genetic information. Such detailed knowledge of the diversity of this soil mesofaunal group will facilitate future biological and ecosystem studies, including those which relate to soil protection and restoration, which require an accurate regional checklist of Collembola; as well as information regarding species distribution and richness estimation in the CFR.

This manuscript exemplifies the utility of using the COI DNA barcode gene to uncover species diversity in poorly studied taxa. It has also been utilized for species discrimination in several other Collembola species (Hogg & Hebert, 2004; Rougerie et al., 2009; Emerson et al., 2011; Porco et al., 2010, 2012, 2014), where it has also served as a useful starting point for the assessment of taxonomic diversity. While studies on species discovery routinely use a single gene (e.g. COI), the use of only a single gene sequence requires cautious interpretation when assessing phylogenetic relationships between species, especially for the deeper nodal relationships. In future, the incorporation of slower evolving nuclear markers are needed to provide a sufficient phylogenetic signal to resolve the deeper levels of evolutionary relationships among taxa, where limited information provided by the COI is most problematic, for a more robust phylogeny (Chapter two). Moreover, there were a few MOTUs exhibiting high levels of intra-specific divergences (i.e. above the 2.50% divergence threshold) with the possibility of incipient speciation, thus the status of these specimens needs to be carefully re-examined in the future (Chapter two). Despite the room left for future improvement, one of the great milestones of this study is its contribution towards the building of a comprehensive Collembola DNA barcode library in both local (Collembola of South Africa, COLSA) and international frameworks (Barcoding Collembola, BCCOL) in the Barcoding of Life Data (BOLD) systems.

Spatial variation in species composition is one of the most important and conspicuous components of biodiversity (Jost et al., 2011). This has been well investigated in the plant species of the CFR, showing exceptionally high levels of turnover between different habitats and among equivalent habitats along geographical gradients (Cowling et al., 1992; Simmons & Cowling, 1996; Goldblatt, 1997). However, the beta diversity of invertebrates in the CFR has seldom been investigated (but see Colville, 2009 - Holpiini monkey beetles), thus it is not known whether they are generally as high as that of the plant species. In this thesis, one of the key findings was the complete or nearly complete turnover of *Seira* species between habitat types (i.e. Fynbos and Southern Afrotemperate forest) and study sites (Figure 4.1), indicating extreme habitat fidelity and geographical localisation (Chapter three). These characteristics may have limited their likelihood of long distance dispersal across unfavourable environmental conditions, as they may have strict preferences for specific, currently unknown ecological factors. It is likely that the CFR comprises many endemic species of *Seira*, and these endemic components may be more susceptible to disturbance than the generalist species, as has been suggested for other Collembola taxa elsewhere (Deharveng, 1996). This emphasises the conservation and evolutionary importance of the CFR for the diversification of this Collembola genus. Several studies have identified this region

as the global and/or South African centre of diversity and endemism for various invertebrate pollinator and plant-specialist groups, such as the lacewings (Sole et al., 2013), weevils (Colville et al., 2014) and Holpiini monkey beetle (Colville, 2009). Determining the hierarchy of abiotic and biotic factors that govern the diversity patterns of *Seira* assemblages at different spatial and temporal scales should be of significant importance in future studies, as well as the ecological and physiological requirements of *Seira*. This information would be useful in developing indispensable new models to foresee their diversity and distribution responses, especially for the endemic and rare species, to our current rapidly changing environment.

It is likely that there are still vast numbers of undiscovered and undescribed species of *Seira*, as many different habitat types and geographical areas remain to be comprehensively sampled in the CFR. Moreover, the richness estimates for Fynbos habitats included in this study suggest that the sampling asymptote for *Seira* has not yet been reached. Therefore more intensive sampling is needed in some of the study sites, as well as broader geographical coverage. Closer attention should also be given to the above-ground vegetation, as there appear to be species composition differences of *Seira* between ground litter and above-ground vegetation (personal observation). Given the exceptionally high beta diversity of *Seira*, and the limitations of this study, I predict that the diversity of *Seira* (representing only one of many Collembola genera) will number in the hundreds. This poses the interesting possibility that Collembola diversity in the CRF may mirror that of the plants.

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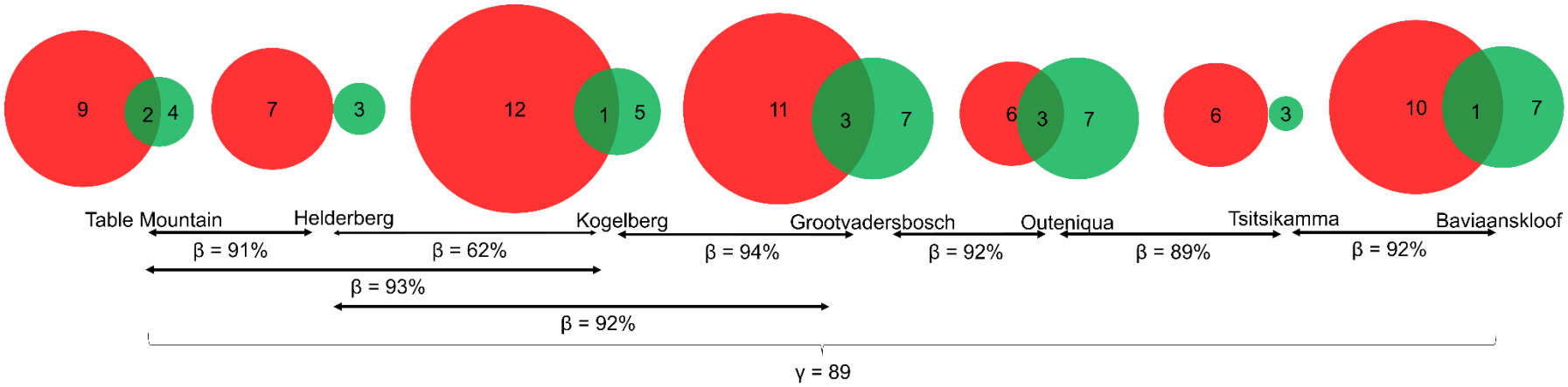


**Figure 4.1.** The local (α), beta (β) and regional (γ) diversity of *Seira* species in both Fynbos and Southern Afrotemperate forest across the seven study sites in chapter three. Venn diagrams showing the shared species between each pair of Fynbos and forest habitat within each study site. The local species richness is shown in each circles. The beta diversities are shown with arrows between some of the sites. Sites without arrow between them indicate complete spatial turnover (i.e. no shared species). The size of the circles is adjusted according to the number of species. The red circles represent Fynbos; the green circles represent Southern Afrotemperate forest.

## Supplementary Figures


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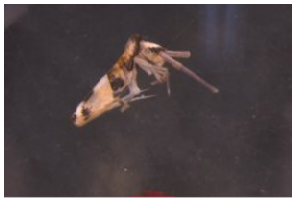
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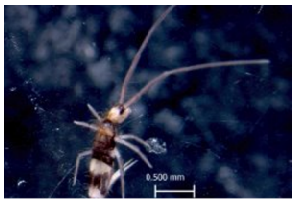

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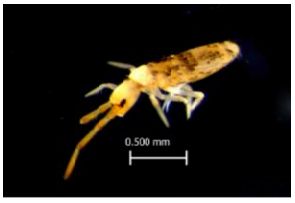

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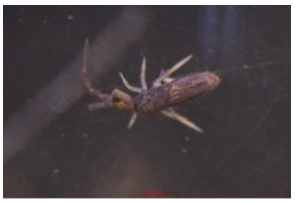
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**Figure S1.** Images of all the different *Seira* MOTUs found in this study (1-91; successfully barcoded). MOTU codes are given and correspond to Table 2.1, and Figure 2.2, 2.4-2.6. The specimens in the last three images (92 to 94) were not successfully sequenced, but they were considered as distinct morphospecies.